

IRRADIATED GAMETES AS A MEANS OF LIMITED GENE TRANSFER IN PLANT BREEDING

A. L. M. Perryman

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Limited Gene Transfer in Plant Breeding**

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ABSTRACT

Following reports that limited gene transfer may be facilitated by making crosses with irradiated pollen, a debate has arisen as to the cause of the observed results. If maternal trends that occurred in the second generation (the M_2) were largely due to the persistence of radiation induced damage, then pollen irradiation would be of little value to plant breeders. But if much of the paternal genome had been eliminated, the method could offer breeders a cheap and simple means of transferring just a few characters from one plant genome to another. By carrying out reciprocal irradiated and control crosses, it was shown in this study with barley that mutational damage is not widespread in the M_2 . However, consistent trends away from the F_2 towards the maternal expression were not observed either. When instead the female gamete was irradiated, moderate shifts to the paternal expression did occur. As trends were for increased vigour, mutational damage is unlikely to have been the cause of these observations. By contrast, when irradiated pollen crosses were made between three varieties of potato, the M_1 and M_2 were consistently lower scoring than the controls. It is suggested that the results may have been different in the two species because polyploids such as the potato may be better able to tolerate radiation damage than diploids such as barley. Gamete irradiation may, therefore, be of little value in polyploid crops. It is recommended that, at least in diploid species, ovule irradiation should be further investigated because not only may it be more effective than its male equivalent, but it may also be easier to perform. Both techniques may be useful in breaking down linkages resistant to conventional crossing.

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PART 1

IRRADIATED POLLEN AS A MEANS OF LIMITED GENE TRANSFER

The Immediate Origins

The First Quantitative Evidence

Feasibility Studies In Crop Plants

- barley
- wheat
- maize
- tomatoes
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The Mechanism Debate

- egg transformation, genomic selection or mutational damage?
- genomic selection in barley
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The Aims Of This Study

The Immediate Origins

Artificial hybridisation and selection have for long been the tools of crop improvement. Even the very earliest farmers practised selection, albeit often unconsciously. They facilitated the development of non-lodging, non-shattering, grain plants, for example, by naturally collecting seed from erect plants whose heads had not shattered [Mayo, 1987].

Although far more recent in the evolutionary time span of crop plants, artificial hybridisation has also been practised for centuries. Once sexual reproduction had been demonstrated in plants, the realisation that crops could be improved through the use of superior parents spread quickly [Forsberg & Smith, 1980]. Fairchild is credited with the production of the first artificial hybrid in 1719 [Roberts, 1929], the technique being thoroughly explored in the two centuries following.

Not only did these studies result in improvements to cultivated plants, they also furthered understanding of the laws of inheritance. As the basic concepts of genetics and reproduction were published, hybridisation followed by selection gradually replaced selection within established populations as the prime method of crop improvement [Forsberg & Smith, 1980], and conventional plant breeding as we know it today was born.

Traditionally, plant breeding has involved the combination of whole genomes, requiring the later segregation of characters and selection of plants in succeeding generations [Lacaderia, 1977; Hadley & Openshaw, 1980]. Two fundamental problems are sometimes encountered with this approach. First, many theoretically attractive genomic combinations are limited by a lack of sexual compatibility between the parental types. And second, the breeding objective is often the transfer of just a single characteristic from one genotype into another; an aim conventionally achieved only by lengthy and laborious backcrossing [Davies, 1981].

Inevitably, plant breeders have sought more rapid and economic ways of transferring specific characteristics into an otherwise desirable genotype. Much attention has recently focused on nucleic acid manipulation as a possible means. Here the ultimate aim is the transformation of plants by the introduction of specific DNA sequences into single cells from which whole plants can be regenerated. Despite rapid progress in this field, it is still an approach fraught with difficulties.

Problems central to this technique include the incorporation of alien structural genes into the host genome and, once there, ensuring their expression [Cohen, 1979]. To achieve the first step alone requires a method for obtaining the DNA to be inserted, a cloning vehicle which is self-replicating in the host, a method of joining the DNA to the vector DNA, a method of introducing the modified vector into host cells, and a method for identifying the cells containing the modified vector [Mayo, 1987]. And even then there may be problems regenerating whole plants from these modified cells. It's hardly surprising that genetic engineering has, as yet, 'contributed little to what is grown for survival or for sale' [Borlaug 1983].

Genetic engineering techniques are not the only ways of transferring specific characters from one genotype to another that have been explored. Another such method, involving an adaptation of conventional sexual hybridisation, has recently been investigated.

As long ago as 1952, the irradiation of pollen was successfully used as a means of overcoming incompatibility [Nishiyama & Iizuka, 1952]. But it wasn't until the late 1970s that the potential of pollen irradiation as a means of limited gene transfer was first recognised.

'Mentor pollen' is compatible irradiated pollen which allows normally incompatible pollen to fertilise an ovule, although incapable of doing so itself. It was during experiments aimed at overcoming intraspecific incompatibility in Nicotiana using such pollen that some unexpected results led Pandey to explore its potential [Pandey, 1975].

When pollen irradiated at 100 Krads was used in crosses involving N. forgetiana and N. alata, a few viable seed were obtained. These gave rise to 24 normal fertile diploid plants which, in general morphology, resembled their maternal parents. However, most plants had the flower colour and/or incompatibility characteristic of the mentor pollen. Following crosses with tester plants of known genotype, 34 plants were recovered carrying 3 S alleles. Triallelic plants of this nature are not produced by any normal form of inheritance, and their appearance was taken as strong evidence that an unusual genetic transfer process was in operation.

Pandey put forward the following process as a possible mechanism of transfer. The contents of the killed pollen tube may have been injected into the egg in a form of 'pseudofertilisation', stimulating the chromosomes of the egg to begin replicating in the normal manner. During this replication cycle, fragments of DNA from the irradiated pollen may have aligned with their homologues in the egg genome. Whereupon, these fragments may have become incorporated into the newly formed chromatid strands either instead of, or in addition to, the original egg segments. Alternatively, diploidy and fertility may have been restored by a second fertilisation by the unirradiated, incompatible pollen.

The First Quantitative Evidence

The first quantitative evidence of specific gene transfer was provided by a series of experiments set up to investigate the possibility of inducing matromorphy (i.e. the production of offspring derived solely from the maternal genome) in Nicotiana rustica. When heavily irradiated pollen was used, offspring showed a general resemblance to their mothers. But, since families derived from them segregated for one or more of the parental differences, they were not strictly matromorphic [Virk et al., 1977]. Subjecting the pollen of N. rustica to near lethal doses of radiation, therefore, appeared to offer a relatively simple means of transferring parts of the DNA from one genotype to another [Mather, 1981]. This possibility was researched both in terms of characters controlled by major genes and those displaying continuous variation [Caligari et al., 1981; Jinks et al., 1981].

Two highly inbred lines were used in this study: V_{27} , homozygous for the recessive markers mophead inflorescence and yellow leaf and stem, was the maternal parent; V_{12} , displaying the dominant characteristics of non-mophead inflorescence and green leaf and stem, was the pollen donor. Both unirradiated pollen and pollen subject to doses up to 20 Krads were used in crosses; the resulting seed giving rise to the F_1 and M_1 generations respectively.

All the F_1 plants were uniform in appearance displaying the dominant characteristics of the paternal V_{12} . The M_1 plants, on the other hand, were highly variable resembling either parent or displaying combinations of both recessive and dominant characters. All plants were selfed; the percentage of M_1 plants setting viable seed falling dramatically with increasing radiation dose.

At all doses the M_2 segregation ratios were significantly different to those of the F_2 . In each case the excess was of maternal types; the populations becoming increasingly maternal with increasing dose. Three height characters scored displayed a similar significant trend. Importantly, plants could be isolated from the M_2 which were similar to the maternal parent but incorporated a single attribute (either major gene controlled or a quantitative character) from the paternal parent.

Close examination of the highest dose group revealed significant differences between families (although they all deviated, at least for some characters, away from the F_2 towards the maternal parent). The authors surmised that not only was just a part of the paternal DNA being expressed, but that it was a different part in families derived from different M_1 individuals. That is, there was no reason to suspect specific regions of the genome were being preferentially affected.

Caligari, Ingram and Jinks [1981] went on to consider possible explanations for their results. The first, that mutations were responsible, would be difficult to reconcile with most mutation studies owing to the directional nature of their results. The second possibility, that a large part of the paternal DNA was inactivated although present in M_1 plants and passed on to M_2 families, could not be ruled out by their findings. The third, and considered most likely explanation, was that only part of the paternal DNA was present in M_1 plants. Since the general appearance and fertility of these plants suggested they were unlikely to be haploid or grossly aneuploid, the maternal genome must have doubled sometime after 'fertilisation' with irradiated pollen. This proposed mechanism is similar to that suggested by Pandey [1975].

Feasibility Studies In Crop Plants

Jinks, Caligari and Ingram [1981] had demonstrated how, by a combination of pollen irradiation and selection, specific paternal characters could be transferred into a pure breeding maternal genotype. Encouraged by the Nicotiana findings, Davis [1981] stressed the need to establish the extent to which this technique could be applied elsewhere.

- barley

Powell, Caligari and Hayter set about investigating the feasibility of this method of gene transfer in the commercially important and self-pollinating crop, Hordeum vulgare [1983]. The cultivar Golden Promise, bearing the recessive traits mildew susceptibility and erect juvenile growth habit, was chosen as the female parent; Magnum, with the dominant characters mildew resistance and semi-prostrate growth habit, was the pollen parent. Whole ears of Magnum were irradiated at doses between 500 and 2000 rads immediately prior to pollination. Fourteen to eighteen days later, the developing caryopses were removed and their embryos cultured on nutrient medium before transfer to compost. First generation material was glasshouse grown; the F_2 and M_2 were sown in the field.

Data from the M_1 generation displayed the same trends as those described by Jinks et al. in Nicotiana rustica [1981]. That is, the higher the radiation dose the smaller the number of viable plants produced and the closer those plants resembled the maternal parent rather than the F_1 . Again, M_2 data revealed the increasing presence of maternal phenotypes with increasing dose. The M_1 phenotype was not, however, a reliable indicator of that of M_2 .

In this first report, height was the only quantitative character considered. And, as the authors point out, it was not possible to tell the extent to which mutational damage contributed to the trend for reduced height in the M_2 . However, for major gene characters Powell et al. argue mutation was unlikely to have played the dominant part since all observed phenotypes derived from parental alleles.

Although this preliminary study was not designed to assess underlying mechanism, parthenocarpy was eliminated as emasculated unpollinated ears did not set seed. In addition, biochemical evidence was supplied which ruled out accidental selfing as the source of at least one of the apparently maternal types.

- wheat

At the same time as the barley study was being conducted, Snape and co-workers were experimenting with pollen irradiation in Triticum aestivum [Snape et al., 1983]. The paternal genotype chosen was the Hobbit (Triticum spelta 5A) single chromosome substitution line. This differed from the maternal parent, Chinese Spring, by several phenotypic and electrophoretic single gene markers, and in a range of quantitative characters. Mature spikes of the substitution line were irradiated with 2,3 and 5 Krads of X-rays immediately prior to pollination. Because abnormal embryo and endosperm development were anticipated, a proportion of embryos were removed at eighteen days and cultured. M_1 plants from all but the lowest dose were sterile, and only two M_2 families could be produced. At all stages of the study, from initial pollination to M_2 meiosis, samples were fixed for cytological examination.

Nuclear abnormalities were observed in both embryo and endosperm following fertilisation by irradiated pollen. Most of the M_1 plants exhibited aneuploidy and signs of structural damage. Further evidence of rearrangement was observed in meiotic preparations, even in those plants apparently normal at the mitotic analysis. By the M_2 , karyotypes appeared much more normal.

In terms of general morphology, the M_2 was far more maternal than the F_2 . But for individual quantitative measures this was not always the case, highlighting one of the difficulties in assessing the effects of pollen irradiation on such characters. For, as the authors acknowledge, unless controls are included to separate the effects of irradiation damage from differential genetic transmission, results may be ambiguous.

By contrast, disturbances of gene frequencies in the M_2 were unambiguously assessed by studying the marker loci. Snape et al. found that the maternal genome was represented to a greater extent than the paternal genome in the M_2 progeny. They suggested this was predominantly due to differential transmission or survival from heterozygous M_1 plants. And, as with earlier Nicotiana work [Caligari et al., 1981], significant heterogeneity between the M_2 families suggested the phenomenon was not specific to any particular locus or chromosome.

As far as a mechanism of transfer was concerned, the authors proposed that true fertilisation took place. But during early development parts of the damaged paternal genome were eliminated, presumably due to failure to participate in mitosis. The M_1 plants which did survive contained most, if not all, of the paternal genome, but it had been reorganised into a karyotype different to that of the maternal genome. Meiosis in these plants was therefore highly irregular. And since progeny from this process were mostly maternal, viable gametes contained a higher proportion of maternal chromosomes.

M_1 meiosis, therefore, appeared to act as a 'meiotic sieve' to limit transmission of paternal genes. Although obviously at variance with the mechanism proposed by Pandey [1975, 1978], this model of limited gene transfer is in keeping with the results he and others [Caligari et al., 1981] obtained in Nicotiana. Indeed, it also fits the findings of Powell and co-workers in barley [1983].

Snape et al. suggested that while pollen irradiation in wheat had only a slight advantage over backcrossing, it was technically quicker and easier to perform. Since wheat is polyploid (and so may allow the transmission of a greater proportion of unbalanced gametes) some 'cleaning-up' would be necessary to extract stable homozygous genotypes.

- maize

Pandey had meanwhile turned his attention to Zea mays [1983]. His earlier egg transformation work in Nicotiana involved the use of lethal radiation doses ('lethal' and 'sublethal' are terms Pandey used to refer respectively to the inability and ability to produce viable seed). The Nicotiana work of Caligari et al. [1981], on the other hand, employed sublethal radiation doses. In this study of eight strains of maize, Pandey used a range of treatments from 5 Krads to 80 Krads covering both types of radiation dose.

No viable seeds were produced at doses higher than 16 Krads. Of the relatively few surviving T_1 (M_1) progeny, more than 50% were phenotypically normal, with about 23% displaying recessive maternal characters to varying degrees. 107 plants derived from a 5 Krad cross were examined cytologically at mitosis: 73% had 20 apparently normal chromosomes, and 26% had 19 chromosomes plus or minus a fragment. Only those plants examined with a full chromosome complement demonstrated any degree of pollen fertility. Two-thirds of these were highly fertile. Of seven plants monitored at meiosis, five appeared perfectly normal, one occasionally produced a single quadrivalent and one displayed very abnormal pairing. No data from the T_2 (M_2) generation were reported.

Pandey's earlier observations using lethally irradiated Nicotiana pollen led him to propound the egg transformation model. That is, that gene transfer was associated with parthenogenetic diploidy induced following pseudofertilisation by lethally irradiated pollen. However, cytogenetical observation of the T_1 progeny in Z. mays indicated that at sublethal doses progeny arose through normal fertilisation. Given this fact, Pandey explored three explanations of his findings in maize.

The first hypothesis, that loss of paternal genes was the dominant cause, was deemed inconsistent with the relatively normal chromosome numbers and behaviour, and the high fertility of the majority of T_1 progeny. Similarly, mutation was dismissed as unrealistic in view of the directional nature of observed trends. The third, and favoured proposal, was that paternal genes were replaced by corresponding maternal genes involving chromosome repair by somatic recombination and gene conversion.

In effect, two almost opposite processes were being advanced for gene transfer involving lethally irradiated pollen and for that involving sublethally irradiated pollen. In the former, the phenomenon was achieved by integration of pollen DNA fragments into the normal egg genome. In the latter, it was brought about by large parts of the pollen genome undergoing substitution by corresponding segments from the egg genome.

Neither model resembles the meiotic sieve proposal of Snape and co-workers [1983]. The sublethal version, which one might expect to be similar, differs appreciably probably because of the conflicting cytological findings in maize and wheat.

- tomatoes

The feasibility of pollen irradiation as a means of limited gene transfer in tomatoes was tested by Zamir [1983]. Pollen from the wild tomato *Solanum pennellii* was irradiated with gamma rays at doses between 5 and 35 Krads, and then used to pollinate a male sterile variety of *Lycopersicon esculentum*. In order to determine the radiation effect on male and female gametes, resulting hybrid plants were used as both male and female parents in backcrosses. Pollen from five M_1 plants derived from the 20 Krad cross, and from five F_1 plants, was used in a backcross with the male sterile *L. esculentum*. And the same hybrid plants were used as female parents in crosses made with *S. pennellii* pollen. Progeny were assayed electrophoretically to determine their genotype with respect to 7 independent enzymic markers.

Pollen irradiation appeared to have little effect on whether or not flowers set fruit. But the number of seeds produced in each fruit did fall sharply with increasing dosage. Of 112 M_1 plants assayed, all but one were normal heterozygotes. And, despite parental differences, there were no differences between the F_1 and M_1 for leaf ratio, stigma exertion or fruit weight either. The proportion of aborted pollen grains did, however, differ and was much higher in M_1 plants than in the control.

Had there been substantial elimination of the irradiated parent genome, then in the backcross produced by pollinating L. esculentum an excess of homozygotes compared to the control backcross would be expected.

This effect was only significant in the progeny of one M_1 plant which did not carry the irradiated pollen allele of Pgm-2. Likewise, in the second backcross (where an excess of heterozygotes would be expected) Pgm-2 was the only locus where a significant excess was observed.

Owing to the relatively minor treatment effects in this experiment, Zamir was unable to resolve the question of mechanism as it related to the successful results in Nicotiana.

- peas

With the aim of repeating the Nicotiana work in a crop plant, Davies [1984] set up experiments with Pisum sativum. The genotypes employed had several linked markers which, were transformation to occur, would be useful in evaluating the size of chromosome fragments transferred. The highest dose at which seed were set was 3,200 rads, and over 95% of M_1 seed germinated. Both F_1 and M_1 plants were morphologically similar and, apart from a few semi-sterile M_1 plants, produced comparable amounts of seed. Results are reported for the 900, 1,200 and 1,800 rad crosses as substantial numbers of M_2 plants were obtained at these doses.

Since the distance between linked markers was known, the expected distribution of phenotypic classes could be calculated. The F_2 was found to agree well with expectation. When M_2 data for each dose was summed and compared to the control, the percentage of progeny in each phenotypic class was not significantly different. However, a different picture emerged when families were considered individually.

The segregation ratio of each allele in each family was calculated. In the F_2 and M_2 (900 rads), a similar proportion of families deviated significantly from the expected segregation ratio at any one of the five loci (7.7 and 5% respectively). But this proportion rose to 11.1% in progenies derived from the 1,200 rad cross, and up to 20% for the highest dose M_2 . On two occasions there was an excess of paternal alleles; there was a complete absence of these alleles on another. In all other instances, however, the excess was of maternal alleles.

When it came to the practical benefits of the technique, Davies supposed there may be some advantage in that linked markers may be separated more easily by irradiation than by recombination. As for increasing the benefit by increasing the dose, the extra effort required to produce seed may balance out any advantage. This is especially true in those plants which have few ovules per ovary; a situation typical of many temperate crop plants.

The author remained uncertain as to the mechanism involved. If Pandey's egg transformation model was in operation, the M_1 would have closely resembled the maternal parent [Pandey, 1975, 1978]. As it was, it resembled the F_1 indicating that a substantial transfer of paternal information from the irradiated pollen had taken place. While lesions induced in the paternal chromosomes appeared not to have impaired expression in the M_1 , they did reduce transmission to the next generation. Davies suggested this reflected either a disturbed meiosis and/or a selective survival of M_1 gametes.

In fact, Davies' theory is essentially the same as the meiotic sieve proposal of Snape et al. [1983], although the uniformity and the relatively high fertility of the M_1 in P. sativum do contrast with the results in wheat. As with the egg transformation model, if Pandey's version of events for sublethally irradiated pollen had taken place, some maternalisation of the F_1 would have been expected [Pandey, 1983].

- peppers

The part pollen irradiation could play in facilitating gene transfer by breaking down gene linkages was investigated further by Daskalov [1984] working with Capsicum. Because gene transfer using conventional breeding techniques is often hampered by a lack of crossing-over within gene linkages, Daskalov suggested the following approach. Pollen is irradiated at doses which, although high, still allow the formation of viable seed. Several studies had shown that structural heterozygosity in one part of the genome increased recombination in the remainder of the genome. That being the case, M_1 plants are selected which possess a high proportion of sterile pollen, and so hopefully have heterozygous chromosomal aberrations. These plants are then used as the male parent for backcrossing.

Anthers of the donor line C-3-1 were irradiated with 1,500 rads of gamma rays, then used to pollinate the recipient genotype, W8, which possessed a number of recessive marker genes. Plants in the resulting M_1 generation were then checked for pollen sterility; 46% displayed normal fertility.

Five plants with more than 80%, and one plant with more than 50%, sterile pollen grains were selected and used to backcross to W8. The segregation pattern of four characters expressed in the resulting backcross generation was assessed and compared with the non-irradiated control.

When data was pooled for all BC-1 irradiated progenies, a highly significant shift towards the maternal phenotype was noted in all but one of the characters examined. Pollen sterility had declined, with 69% of plants now normally fertile.

Daskalov concluded that pollen irradiation combined with the selection of M_1 plants with a high percentage of sterile pollen for backcrosses may reduce the time it takes to achieve desired gene transfer. Irradiated pollen could be used again in the crossing programme should undesirable linked genes persist.

The Mechanism Debate

All the studies so far examined reported a trend towards the maternal parent in the generations following fertilisation with irradiated pollen (albeit to varying degrees). How exploitable this phenomenon would be, remained to be seen. And the mechanism behind the observed trends was still in question.

- egg transformation, genomic selection or mutational damage?

To try and find out which, if any of the proposed mechanisms was the right one, Werner, Dunkin, Cornish and Jones [1984] undertook a cytological examination of both inter- and intraspecific M_1 progeny in the Nicotiana genus. By crossing two species with different chromosome numbers (N. rustica $2n=48$, and N. paniculata $2n=24$), Pandey's transformation model could be tested. And by repeating the intervarietal cross ($V_{27} \times V_{12}$) carried out by Caligari et al. [1981], further explanation of the earlier observations might be possible. Efforts were concentrated on a dose of 20 Krads.

Of 56 M_1 progeny from the interspecific cross examined, half possessed karyotypes indistinguishable at mitosis from that of the normal hybrid. The other half showed evidence of chromosomal damage, chromosomal loss, or both. Phenotypically, no trend towards the maternal parent was apparent. But a number of plants did display morphological aberrations, these tending to be more extreme in plants where chromosomes were damaged or missing.

Additional evidence of chromosome damage was supplied from the meiotic analysis, where 3 out of 9 plants consistently produced quadrivalents revealing reciprocal translocations not apparent at mitosis.

Of 35 M_1 plants resulting from the intervarietal cross, only 5 had apparently normal karyotypes at mitosis. Some plants resembled the normal F_1 phenotype, but Werner et al. report that many displayed the expected deleterious effects of chromosome damage and aneuploidy. Once again, multivalents were observed in the meiotic configurations.

Had Pandey's transformation mechanism of gene transfer been in operation, the progeny of both crosses would be expected to have the normal maternal karyotype. This was definitely not the case in the interspecies cross where plants all had approximately the triploid number of 36 chromosomes. Neither did the progeny of either cross resemble the maternal phenotype. In addition, most plants had reduced fertility (which together with the lack of maternal resemblance rule out Pandey's sublethal model too [Pandey 1983]). Instead, each M_1 appeared to be the result of conventional fertilisations, albeit with damage induced by radiation.

While many plants of the intervarietal M_1 differed morphologically from the corresponding F_1 , Werner and co-workers believed them merely aberrant, displaying typical deleterious effects of mutations, deletions, and aneuploidy. Since it seemed likely that some of this chromosomal damage was inherited by the M_2 , the authors maintained it was this that caused at least part of the maternal trends reported by Caligari *et al.* [1981]. The trend for reduced height, for example, may simply have reflected a loss of gene function as a result of radiation damage.

Whether or not post-meiotic selection as proposed by Snape *et al.* [1983] played an important role could not be determined from this study. However, in Werner *et al.*'s opinion it seemed unlikely that the meiotic process itself acted as a selective agent. Instead, some of the mutations and deletions carried by the paternal chromosomes may have been lethal in gametes or resulting zygotes. If this, rather than radiation damage, was the case, true maternal trends would result regardless of the direction of the cross.

The authors concluded that if, as they suspected, the reported observations were merely conventional effects of radiation, irradiation of pollen was unlikely to have a useful application. If, on the other hand, gametophytic selection was responsible, such selection would have to be very strong to make this technique of use in plant breeding.

- genomic selection in barley

As a follow-up to the study of the behaviour of major gene characters following crosses with irradiated pollen in barley [Powell et al., 1983], Caligari, Powell and Hayter discussed the quantitative data from the same experiment [1984]. Four characters were measured in the field grown M_2 : neck length, tiller number, grain number and thousand grain weight.

For three characters where the mean of the F_2 was distinguishable from those of the parents, the M_2 data was consistent with a maternal trend increasing with dose. When grain number (a character directly connected with fertility and so particularly susceptible to radiation damage) was examined, the mean of the highest dose M_2 was actually greater than the F_2 mean, although not significantly so.

If genomic selection did not occur between the M_1 and M_2 in barley, then these changes must have reflected the effects of radiation damage. Caligari et al. therefore maintained that even if no phenotypic differences exist between the parents and the F_2 means, a trend in mean M_2 phenotype should have resulted because of dose effects. If, on the other hand, genomic selection was taking place, the relative contribution of the original paternal parent would have decreased. So, the difference between the F_2 and the M_2 mean should have closely resembled the difference between the F_2 and the maternal mean (rather than that between the F_2 and the paternal mean).

For all quantitative characters scored, the similarity between F_2 -GP and F_2 - M_2 was striking. In the case of tiller number and thousand grain weight, the M_2 means were lower than those of the F_2 , which could have reflected elimination of paternal material or radiation damage. However, deleterious effects would not be expected to be so dependent on the F_2 -GP difference, and instead would produce an excess of very low scoring phenotypes. This was not the case for either of these characters.

The authors concluded that radiation damage was unlikely to be largely responsible for their observations. Instead, they believed the paternal genome was preferentially eliminated during production of the M_2 giving rise to a shift towards the maternal phenotype.

- **mutational damage in Nicotiana**

In order to clarify the extent to which irradiated paternal chromosomes were transmitted to the progenies of M_1 plants in Nicotiana, Werner and Cornish [1984] continued their cytogenetic analysis into the second generation. By including reciprocal backcrosses they hoped to detect not only the occurrence of selection, but also, if it was present, whether it differed in intensity between the two gametophytes.

V_{27} and V_{12} were crossed reciprocally with pollen irradiated at a dose of 20 Krads, and the resulting generation was backcrossed reciprocally to V_{27} . Four M_1 plants (two from each cross) were randomly selected for this analysis; 30 progeny from each M_1 ($10 V_{27} \times M_1$, $10 M_1 \times V_{27}$, $10 M_2$) were then grown in the glasshouse.

All of the chosen M_1 plants were aneuploid, two being monosomic and two trisomic. As well as these numerical abnormalities, several structurally altered chromosomes (which must have been paternal in origin) were identified. Assuming regular and random meiotic configurations, and in the absence of gametophytic selection, half the gametes would be expected to be euploid and half to have either one too few or one too many chromosomes. Likewise, half the gametes would be expected to carry a given structural marker, and half its normal homologue.

The mitotic survey of BC,RBC and M_2 progenies revealed that 83/120 individuals carried one or more numerical or structural aberration, a result Werner and Cornish declared striking. However, 69% of plants in the backcross generations, and 37.5% of the M_2 , were euploid, significantly more than the respective 50% and 25% expected. Of the six structurally altered marker chromosomes, two were inherited normally, one was preferentially transmitted and three were selected against. When all aberrations were considered together, significantly more BC than RBC plants had 48 normal chromosomes. Selection was therefore stronger in the male gametophyte.

Because they had used similar materials, doses and techniques, Werner and Cornish deduced from their results that about 80% of the M_2 plants assessed by Caligari et al. [1981] were carrying sizeable chromosome aberrations. This, they claimed, strengthened their earlier assertion [Werner et al., 1984] that trends observed in N. rustica following pollination with irradiated pollen reflected, at least in part, loss of vigour due to the deleterious effects of radiation damage.

While selection against radiation induced damage did occur, Werner and Cornish maintained that it was of insufficient intensity to account for the magnitude of the maternal trends reported by Caligari et al. [1981]. They calculated that if selection alone were responsible, the average frequency of maternal alleles controlling height would have had to increase to 82% to produce the final height recorded by Caligari et al. in their 20 Krad M_2 . The actual figure obtained in this experiment, however, was only 67%.

Thus they believed a more probable explanation of the apparent maternal phenotype in N. rustica was the high frequency with which aneuploidy and deletions persisted in the M_2 generation. It is worth noting, however, that the effect of dose will vary with the state of the pollen, the method of presentation and so forth. A comparison of the survival numbers obtained in both studies suggests that the 20 Krad dose Werner and Cornish used may have been effectively lower than that employed by Caligari and co-workers.

- cytological evidence in other crops

If Werner and Cornish were correct, pollen irradiation would be of little value as a tool for plant breeders. If, however, an effective gametophytic selection mechanism was in operation its potential would be far greater. In order to contribute to the understanding of the mechanism(s) involved, Borrino, Caligari, Powell, McNaughton and Hayter [1985] conducted studies within three crop groups: brassica, potato and barley.

Interspecific irradiated crosses between Brassica napus ($2n=4x=38$) and B. campestris ($2n=2x=20$) produced M_1 plants with a chromosome number the same as, or close to, that of the triploid F_1 ($2n=3x=29$). Just as interspecific crosses in Nicotiana had done previously [Werner et al., 1984], these results substantiated the notion that irradiated pollen derived material is hybrid rather than parthenogenetic in origin. About half the M_1 plants sampled were hypoploid and structural re-arrangements were also frequent; findings common to M_1 generations in wheat [Snape et al., 1983] and in Nicotiana [Werner et al., 1984]. Preliminary screening of intervarietal potato crosses (Solanum tuberosum $2n=4x=48$) also revealed irradiation induced aneuploidy. When it came to barley however, the results were quite different.

Triumph was the cultivar chosen as the female parent; Tweed was the pollen donor subjected to radiation doses between 750 and 1500 rads. None of the resulting M_1 plants examined were aneuploid, and 9/13 showed no evidence of structural rearrangement at all. Borrino et al. considered it likely that the type of damage which generates aneuploid gametes did occur in barley pollen. The absence of aneuploidy could be explained by the lack of tolerance of hypoploidy in true diploids, together with the rarity of radiation induced generation of hyperploids.

By the M_2 there was even less evidence of structural abnormality. Moreover, M_1 individuals which had had reduced seed sets but no sign of structural change (suggesting the presence of cryptic chromosomal damage) produced progeny which had normal levels of fertility.

Although only a preliminary investigation, these findings may offer some explanation as to why observations of the M_2 in barley differ substantially from corresponding results in Nicotiana.

- evidence from irradiated selfs in barley

By carrying out a selfing programme using irradiated pollen in highly inbred barley cultivars, Powell and Caligari [1985] hoped to throw more light onto the genomic selection/mutational damage debate. Because if, as Werner and Cornish [1985] suspected, mutational damage played the major part, then some sort of variation would be expected in the second generation following selfing with irradiated pollen.

Golden Promise and Magnum were chosen as the cultivars used in earlier experiments. Pollen from both was irradiated at doses between 500 and 1500 rads, and unirradiated control selfs were also produced. The second generation was grown in the field where two major gene characters, mildew resistance and growth habit, were scored. After harvest, a random sample of plants from each row was scored for four characters displaying continuous variation.

No evidence of any segregation was found. Neither were there any significant differences for any of the quantitative characters examined. In previous experiments [Powell et al., 1983; Caligari et al., 1984], pollinating Golden Promise with irradiated Magnum pollen resulted in an M_2 generation with a preponderance of Golden Promise characters. Were this to be due to the phenomenon described by Werner et al. [1984], Magnum alleles would have been largely inactivated by irradiation resulting in an expression similar to that of the counterpart alleles in Golden Promise. Powell and Caligari maintained that similar inactivation of Magnum should have occurred in the irradiated selfs. But the then expected shift towards the expression of recessive characters (so mimicking Golden Promise) clearly did not happen. So the authors held to their earlier conclusion and stated that in barley, damage induced by irradiating pollen cannot adequately account for observed maternal trends in later generations.

- quantitative and qualitative evidence in Nicotiana

Cytological investigations in Nicotiana provided evidence that both mutational loss and genomic selection played a part in determining M_2 phenotype. In order to assess the relative importance of these factors, Werner and Cornish compared M_2 generations from reciprocal crosses [Werner and Cornish, 1985; Cornish and Werner 1985].

Pollen from the V_{12} and V_{27} varieties of Nicotiana rustica was irradiated with 20 Krads of gamma-radiation and used to pollinate the stigmas of both varieties to provide the reciprocal crosses and the selfs of both parents. As well as these crosses, a sample of M_1 plants was used in reciprocal backcrosses with V_{27} . The major gene characters scored were ovary colour, flower colour and inflorescence morphology, believed to be under the control of 1, 2 and 3 genes respectively.

Generally speaking, the irradiated selfs closely resembled their respective parents, and the M_1 hybrids were similar to the F_1 . Both, however, were more variable than their unirradiated counterparts. 21% of plants from $V_{27} \times V_{12}$ failed to inherit a functional allele for ovary colour from V_{12} and 1.1% lacked either of the paternal alleles for flower colour. By analysing the three generations derived from the M_1 of $V_{27} \times V_{12}$, Werner and Cornish were able to separate the two effects of radiation. Significantly fewer than expected black ovaried plants were found in all three generations. However, when non-segregating families were removed, two of the deviations were reduced to a non-significant level. The authors deduced these two generations were displaying the effects of mutational loss, while the residual deviation in the third reflected maternal selection in the pollen.

Interestingly, when generations derived from irradiated selfs of the dominant variety, V_{12} , were examined, strong evidence of selection was found. Only three non-black plants were recovered in just two RBC families. Werner and Cornish considered their rarity in these two families, and their absence in equivalent BC families, indicated the occurrence of strong selection in the M_1 gametophytes and/or the resulting zygotes. Presumably, the absence of variation in the M_2 for this character (and a lack of variation in all qualitative characters scored in all other generations derived from V_{12} irradiated selfs) adds weight to this interpretation. Not only that, it is also consistent with the experience of Powell and Caligari [1985] in barley.

Deviation in favour of maternal alleles was seen for all characters. But as the average frequency with which alleles of maternal origin were transmitted from the M_1 to the second generation was 0.55, only mild selection was indicated. Werner and Cornish calculated this frequency for other published studies and, with the exception of the data of Powell et al. [1983], produced very similar estimates.

As well as the major gene traits, nine quantitatively inherited characters were scored in this experiment [Cornish and Werner 1985]. Irrespective of the direction of the cross, the consistent trend was towards a reduction in vigour. So, plants tended to be shorter, to have smaller leaves and flowers, and to come into flower more slowly. In some parental combinations (such as the one used by Caligari et al [1981]) this trend appeared maternal, but results from the reciprocal crosses suggested this was merely coincidental.

When the BC and RBC generations were compared, further information about the effects of radiation was obtained. Unless maternal effects had occurred or the rates of transmission of damaged chromosomes differed in the male and female gametophytes, the genetical expectations of these generations would have been identical.

For all characters measured the RBC was lower than the BC. Maternal effects should be greatest in characters scored early on in the growing season rather than those scored later as was the case here. So Cornish and Werner thought it more likely that selection was stronger in the M_1 pollen that produced the BC generations than in the ovules which gave rise to the RBC generations (where, they stated, it may not be acting at all).

In this, the last of their reports on pollen irradiation in Nicotiana, Cornish and Werner asserted that while disturbed segregations of major genes were observed in favour of the maternal alleles, such effects were slight and achieved only at the expense of considerable deleterious radiation damage.

- attempted egg transformation in other crops

Sanford, Chyi and Reisch conducted experiments to see if egg transformation, as described by Pandey in Nicotiana, could be extended to other genera. Despite screening a total of 87,000 potential transformation events in tomato [Sanford et al., 1984a], in maize [Sanford et al., 1984b], and in pea, rapeseed and apple [Chyi et al., 1984], they failed to find any transformants.

Chyi and Sanford [1985] then set about confirming Pandey's observations in Nicotiana, screening for 1,594 potentially detectable transformation events. A very low frequency of unexpected progeny were produced, but the authors report these results were not repeatable and appear to have arisen by mechanisms other than transformation. They concluded that Pandey's previous observations of high frequency egg transformation were not reproducible.

In a critical appraisal of Chyi and Sanford's observations, Pandey [1986] explained some of their unexpected results in terms of transformation events. Whatever their cause, however, the frequency of such events still remained low. Furthermore, attempts by Cornish and Werner [1985] to repeat Pandey's experiments also failed. Pandey's own egg transformation results, therefore, provide the only supporting evidence for his theories.

THE AIMS OF THIS STUDY

As the review of literature on the subject demonstrates, the most extensive studies using irradiated pollen have been in barley and Nicotiana. Unfortunately these pieces of research have produced conflicting results, and so the problem of underlying mechanism has yet to be resolved. Until the relative importance of genomic selection and mutational damage has been established, the precise value of pollen irradiation in plant breeding will not be known.

Data from reciprocal irradiated crosses in Nicotiana suggested mutational damage was the key factor. But in barley, where results had been all together more promising, similar reciprocal crosses had not been performed. These, then, were to form the first part of this study.

Additionally, if irradiating pollen produced an excess of maternal phenotypes in the M_2 generation, could a corresponding paternal trend be induced by irradiating ovules? Not only would the mechanism be of interest, so too would the practicalities of the technique. Because if, as seems likely, ovules are more radiation-tolerant than pollen grains, production of viable seed from which to generate the M_1 may be substantially easier.

Practical implications were also important in the choice of the potato, Solanum tuberosum, for the third investigation using irradiated pollen. Because of the vegetative nature of this species, M_2 plants with the desired characteristics could simply be multiplied. So, in the case of potato, infertility beyond the M_1 generation would not be a problem.

By working with two quite different crops, the potato and barley, it was hoped that a useful assessment of pollen irradiation as a means of limited gene transfer in plant breeding programmes could be made.

PART 2

RECIPROCAL IRRADIATED POLLEN CROSSES IN BARLEY

Introduction

Experimental Design

Method

- crossing procedure
- the glasshouse stage (M_1)
- cytological sampling
- the field stage (M_2)
- scoring qualitative and quantitative characters

Results

- the first generation
- cytology
- quantitative characters in the second generation
- qualitative characters in the second generation
- linkage study: qualitative characters
- linkage study: quantitative characters
- linkage study: qualitative and quantitative characters

Discussion

- the first generation
- cytology
- quantitative characters
- qualitative characters
- linkage

Introduction

Barley (*Hordeum vulgare* L. $2n = 2x = 14$) is an important small-grained cereal crop, the cultivation of which dates back at least as far as 7000 BC. It is principally used converted to animal feed and as a human foodstuff, or as malt for food and beverages. Although only fourth after wheat, rice and maize in the world cereal production rankings, barley is cultivated over a wider range of environments than any of these. While spring barley is predominantly grown, winter types are being increasingly cultivated in regions where winters are mild.

The contribution plant breeding has made to barley production is demonstrated by the steady increase in UK yields since the war (Figure 1).

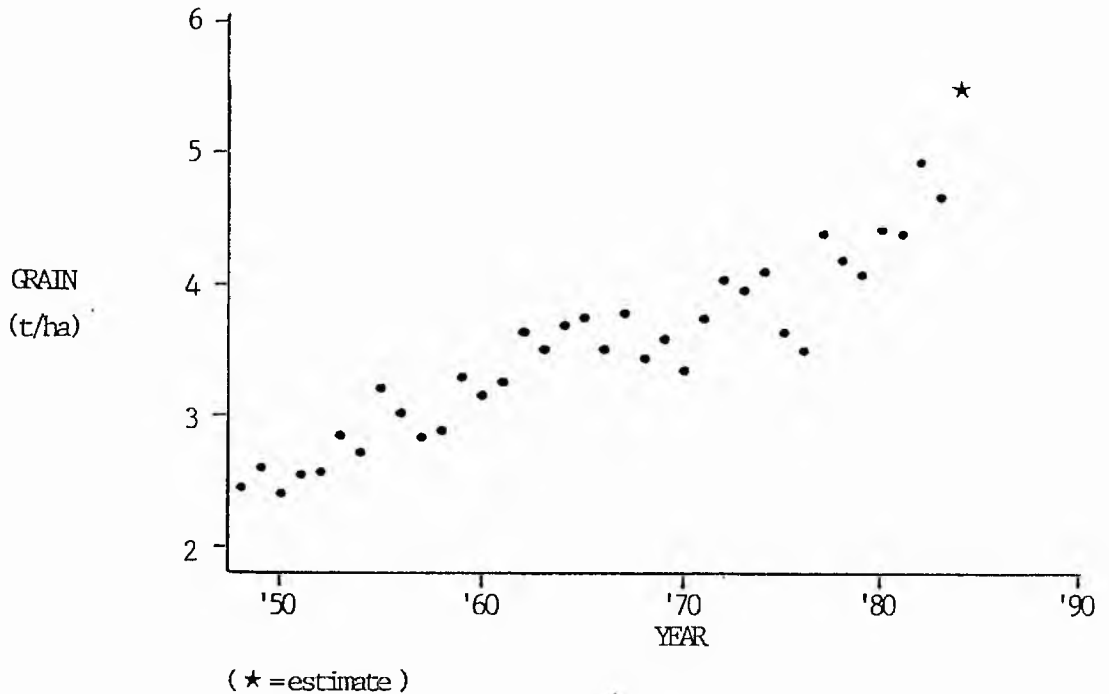


Figure 1: UK barley yields [Jenkins, 1985]

Despite the obvious success plant breeders have had with barley, breeding remains an inexact science relying as it does on the skill of the breeder in choosing parents and in selecting progenies. As an inbreeding crop, individual barley varieties are pure lines. So the variability upon which to practice selection must be generated by making crosses between lines which differ for the characters of interest. Where the character is described by a large number of segregating alleles, large populations must be grown because the frequency of the desired genotype will be low. Furthermore, early selections will be amongst lines which are largely heterozygous. Dominance and heterozygous interactions may then obscure phenotypic expression that selfing later reveals.

In order to improve the selection process in barley, breeders have tried deferring selection until later generations. In practice, this requires the rapid production of homozygous lines which has been achieved by both double haploid production [Reinbergs et al., 1975], and by single seed descent [Snape and Riggs, 1975]. But even if these methods are employed, many lines still have to be produced in order to preserve enough variation to enable successful selection to take place.

Unfortunately, the potential to transfer specific DNA sequences using genetic engineering techniques is, as yet, unrealised in barley. Part of the problem is a lack of suitable vector; the Ti plasmid of Agrobacterium tumefaciens is a useful plant vector, but the bacterium does not so readily attack monocotyledons and so is less successful when used for the transformation of cereal plants. Not only is the vector a problem, but the capacity to regenerate whole plants from single cells is also limited in monocotyledonous plants, although success has been reported recently in maize and rice.

In the absence of successful genetic engineering techniques in barley, the pollen irradiation work of Powell et al. [1983] raised hopes that a relatively cheap and simple means of facilitating limited gene transfer in barley had been found. The experiment reported here was set up in 1983 in order to help clarify the value of this technique in barley breeding.

Experimental Design

Reciprocal irradiated crosses and corresponding control crosses were carried out using two highly inbred lines of spring barley. The cultivar Golden Promise [GP], widely grown in Scotland for its early ripening and good ear retention, was used in crosses with the marker stock S138. These two lines differ in a number of quantitative and qualitative characters; the major gene differences utilised in this experiment are detailed below.

S138

fs - fragile stem

r - smooth awn

s - short rachilla hairs

B - black seed

Ert - tall

GP

Fs - non fragile stem

R - rough awn

S - long rachilla hairs

b - white seed

ert - dwarf

Crosses were made according to the following schedule:-

GP x S138	0 rads	- F ₁
	500 rads] - M ₁
	1000 rads	
	1500 rads	
	2000 rads	

S138 x GP	0 rads	- F ₁
	500 rads] - M ₁
	1000 rads	
	1500 rads	
	2000 rads	

The F₁ and M₁ were raised in the glasshouse and selfed to produce the F₂ and M₂ which were field grown. Each generation was scored for both quantitative and qualitative characters.

Method

- crossing procedure

Samples of both parents were emasculated using the 'egg-topping' method described by Pope [1944]. In general, awns of spikes ready for emasculation had emerged slightly, with the edges of the flag leaf sheath just beginning to separate (Figure 2a). The flag leaf sheath was removed at the level of the first node of the rachis, so offering some support to the neck of the ear (Figure 2b).

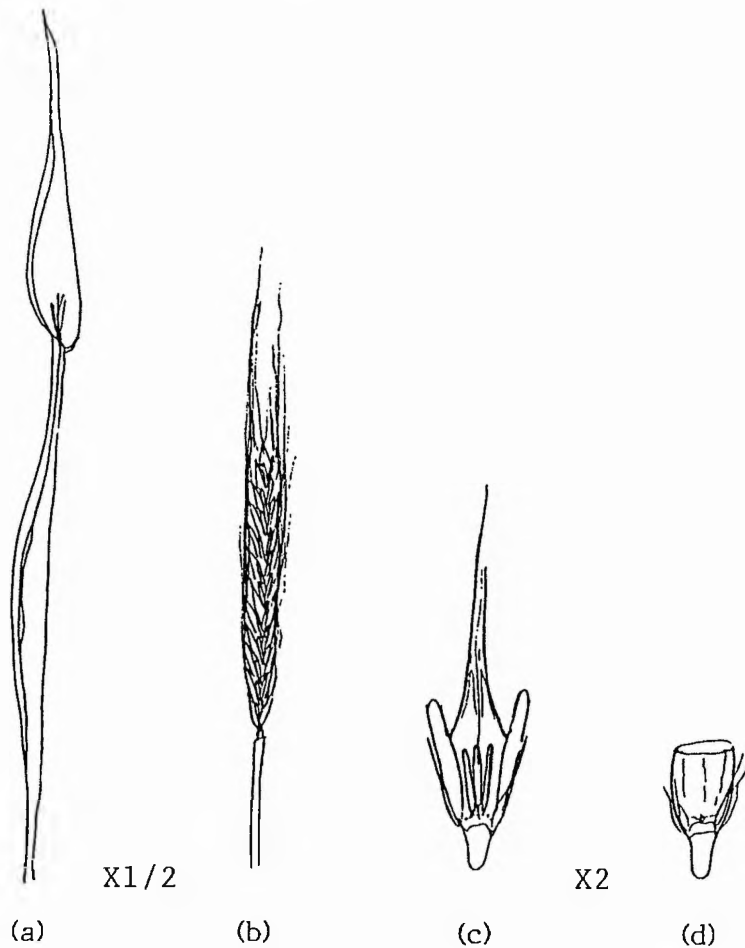


Figure 2: Various stages of emasculation in barley

The three anthers in each floret were usually visible through the lemma (Figure 2c). Since lateral florets sometimes bear viable pollen, these were removed. The palea and lemma were then clipped just above the top of the anthers so that these too could be removed (Figure 2d).

Following emasculation, spikes were covered with clear bags and labelled with the date and their genotype. Some two to three days later they were ready for pollination.

Whole ears of Golden Promise and S138 (slightly more advanced than those suitable for emasculation) were removed and irradiated immediately prior to pollination. A Cobalt 60 source at the Western General Hospital, Edinburgh, was employed to deliver gamma radiation doses of up to 2,000 rads (this being the highest dose used successfully in barley by Powell *et al.* [1983]). Unirradiated control ears were also collected.

Back in the glasshouse, florets were clipped and where necessary anther extrusion was encouraged by placing spikes under a lamp. A plastic tube was then held in place surrounding the emasculated spike, and the pollen parent inserted. Pollination was effected by twirling one ear around the other. Each time pollen parents were changed, the pollinating tube was cleaned with alcohol.

Immediately after pollination, spikes were covered with brown paper bags, and their labels completed with the date and male parentage. Embryo development was encouraged by applying gibberellic acid (GA_3) at 75 ppm to spikes 24 and 48 hours after pollination. At least 30 days later, spikes were harvested and dried. Ears were hand-threshed and seed stored in a refrigerator.

- the glasshouse stage (M_1)

Preliminary investigations indicated that unsterilised irradiated hybrid seed may be subject to fairly high losses when sown in dishes due to infection. For this reason, seed was surface sterilised in 30% hypochlorite solution for several minutes and rinsed in sterile water prior to sowing on moist filter paper in petri dishes. Germination was also improved by the removal of the half of the seed coat that remained following crossing.

After sowing, seeds were incubated in the dark at 20 °C until germination occurred. Resulting seedlings were transferred to Jiffy 7 peat blocks before being potted-on into 12cm plastic pots containing John Innes No. 2 compost. The M_1 and F_1 plants, together with their parents, were grown in a randomised block in the glasshouse during the early summer of 1984.

Pots were placed on a sand bed, the water table being about 5cm below the surface. Temperatures generally did not exceed 20 °C, and a 16 hour photoperiod was maintained using sodium lighting whenever necessary. Individuals were allowed to self-pollinate and as they ripened the water table was lowered until, approximately two weeks before harvest, the water supply was disconnected entirely.

At maturity, plants were scored for major gene characters in addition to height, ear length, the number of seed on the main tiller and the bulk seed number. Each plant was hand-threshed and the resulting seed stored in a refrigerator.

- cytological sampling

A number of F_1 and M_1 plants from the GP x S138 cross were cytologically screened at mitosis and at meiosis. Root tips were collected from plantlets growing in Jiffy 7s, and pre-treated in a saturated solution of alpha-bromonaphthalene for 4 hours before being fixed in glacial acetic acid. Pollen mother cells were obtained from inflorescences fixed in 3:1 alcohol: glacial acetic acid. For both mitotic and meiotic preparations, samples were hydrolysed in 1N HCL at 60 °C for 10 minutes and stained in feulgen.

- the field stage

Each F_2 and both parents, together with M_2 families which had more than 25 seeds, were field-grown in a replicated randomised complete block design at Pentlandsfield, Midlothian, in 1985.

The number of families raised for each treatment is given below:

F ₂ GPxS138	10	F ₂ S138xGP	10
M ₂ -500rads	25	M ₂ -500rads	18
-1000rads	12	-1000rads	7
-1500rads	1		

Each family was represented in each of two blocks by a row of up to 20 plants. The plants were 5cm apart within rows, and there was a 20cm gap between rows.

Spring wheat was grown as the first and last row of each block, and as a guard plant at both ends of each row. It was also used to fill the rows of those M₂ families with insufficient seed to complete both replicates. The experiment was netted to prevent bird damage.

- scoring qualitative and quantitative characters

Because individuals homozygous for the fragile stem marker were fairly delicate, scoring only took place during and after harvesting to avoid damaging plants as they grew. An unfavourable long-term weather forecast necessitated an early harvest.

the quantitative characters

i height

Mature plant height was measured from the base of the plant to the top of the tallest tiller.

ii ear length &

iii awn length

The length of the ear on the main tiller was measured together with its awn length

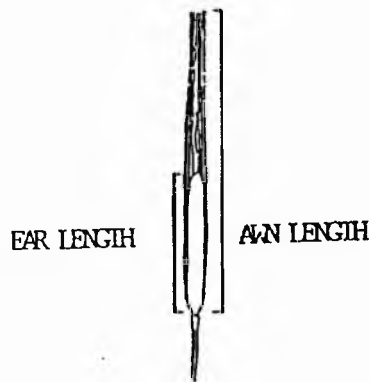


Figure 3: Measuring ear and awn length

iv tiller number &

v green tiller number

The number of mature, fertile, tillers on each plant was counted. As the plants ripened, occasional weak new shoots appeared which were generally sterile. Because of the slightly early harvest, some of these shoots were still green. Since these may/may not have been fertile, they were counted separately.

the qualitative characters

i non-fragile (Fs) vs fragile (fs) stem

When plants were immature, the difference between fragile and non-fragile stemmed plants was relatively easy to detect; homozygous fs plants snapped in two very easily, while those carrying the dominant Fs allele did not. For this very reason, plants were not scored until they were being harvested when the difference between fragile and non-fragile stem was not quite so easy to distinguish.

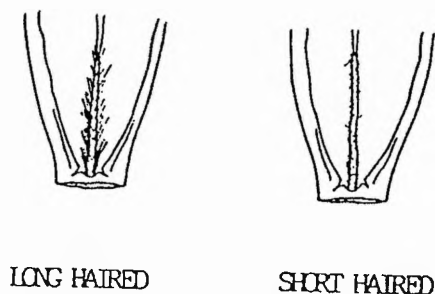
ii rough (R) vs smooth (r) awn

The difference between rough and smooth awns could be felt by rubbing the awn backwards and forwards between the fingers. In cases of uncertainty, the classification was confirmed by examining the awn under a stereo microscope.

iii black (B) vs white (b) lemma and pericarp

iv long (S) vs short (s) rachilla hairs

This character was scored with the help of a stereo microscope.



X 4

Figure 4: Ventral view showing rachilla hair type

v tall (GP Ert) vs dwarf (GP ert)

Golden Promise possesses an erectoides dwarfing gene. (Shortness of straw is the most important character limiting lodging in barley [Stanca et al., 1979], and dwarfing genes of this kind have therefore been used extensively [Thomas et al., 1984]). While the GP ert gene cannot be directly identified, it can be distinguished using quantitative data.

In this experiment the erectoides gene was classified on the basis of height (as used by Thomas and co-workers), and on the ratio of ear to awn length [Powell, personal communication]. The frequency distribution of height in the F_2 population is presented in Figure 5. Plants 63cm or less in height, whose awn length was less than or equal to twice the ear length, were classified as possessing the GP ert gene.

GP ert : HT \leq 63cm &

AL \leq 2 EL

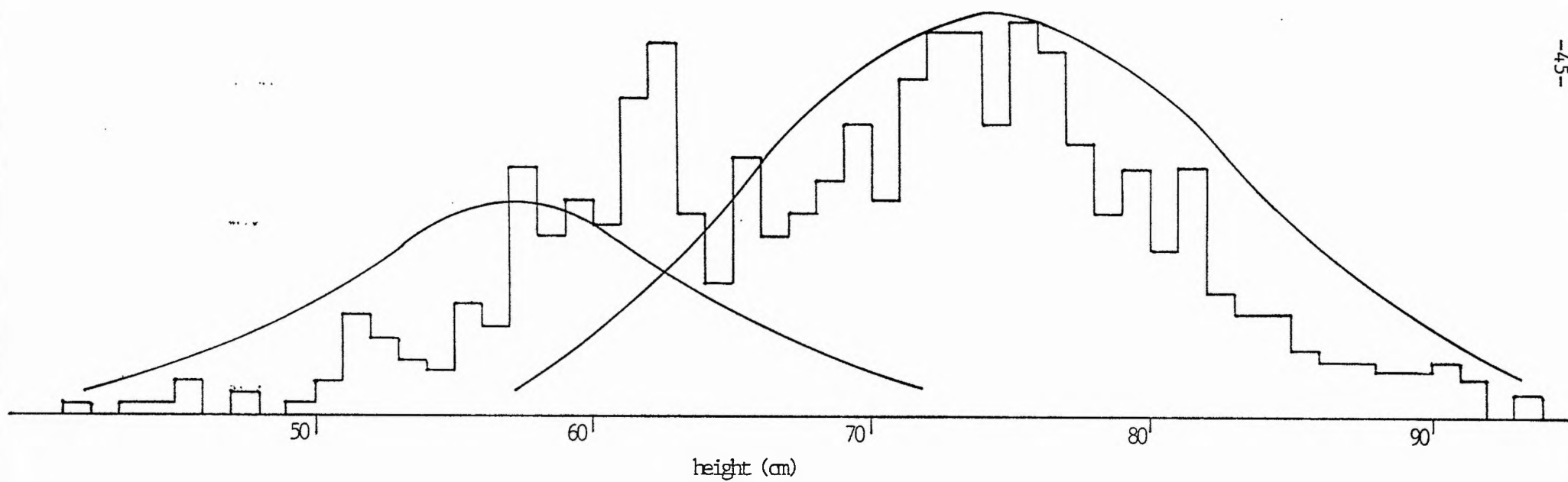


Figure 5: Distribution of height in the F₂ population

Results

- the first generation

Data from the F_1 confirmed the dominance relationships. Not only did all F_1 individuals have rough awns, long rachilla hairs, non-fragile stems and black seeds, so did all M_1 plants too.

A summary of the quantitative data obtained from these plants is given in Table 1. Whichever the direction the cross had been made, M_1 plants were generally taller than their F_1 counterparts. This was most marked in cases where S138 had been the maternal parent. For ear length, M_1 means hovered either side of their respective F_1 means and no pattern was discernible. The number of seed set, however, was consistent with a trend, seed set falling as radiation dose increased.

- cytology

A summary of cytological observations is presented in Table 2. All F_1 plants examined at mitosis had a full complement of chromosomes ($2n = 2x = 14$), and the normal karyotype. Meiotic configurations were also normal with only seven bivalents present.

Of 27 M_1 -500 plants sampled, all were euploid but three displayed aberrant karyotypes. 1 of the 3 was again examined at meiosis when occasional quadrivalents were observed. Abnormal meiotic configurations were also seen in 2 out of 9 plants apparently normal at mitosis.

All M_1 -1000 plants were euploid, but the frequency of mitotic aberrations rose to 7 out of the 17 plants sampled. One of these re-examined at meiosis produced quadrivalents; another was sterile. Of 2 plants apparently normal at mitosis, 1 produced quadrivalents while the other again appeared normal at meiosis.

At the 1500 rad dose, 1 of 4 plants examined was aberrant in karyotype, although it did possess 14 chromosomes. This plant died before flowering. The only plant recovered at the 2000 rad dose was euploid, karyotypically aberrant, and later found to be sterile.

Table 1: Summary of quantitative data from the F₁ and M₁ generation

genotype	number of plants	height (cm) <u>±</u> SEM	ear length (cm) <u>±</u> SEM	number of seed set <u>±</u> SEM
GP X S138	60	68.15 <u>±</u> 1.21	8.42 <u>±</u> 0.20	131.00 <u>±</u> 7.80
-500rads	40	70.18 <u>±</u> 1.60	8.00 <u>±</u> 0.29	74.33 <u>±</u> 9.31
-1000rads	14	72.07 <u>±</u> 4.01	7.36 <u>±</u> 0.61	93.15 <u>±</u> 16.81
-1500rads	4	75.50 <u>±</u> 5.69	8.25 <u>±</u> 1.11	64.50 <u>±</u> 44.19
-2000rads	1	81.00	9.00	0.00
S138 X GP	10	72.00 <u>±</u> 2.75	8.10 <u>±</u> 0.35	122.10 <u>±</u> 16.54
-500rads	21	81.57 <u>±</u> 1.77	8.05 <u>±</u> 0.24	89.33 <u>±</u> 11.85
-1000rads	9	80.89 <u>±</u> 4.51	8.22 <u>±</u> 0.68	44.22 <u>±</u> 10.62
-1500rads	1	70.00	1.00	1.00

Table 2: Cytological observations in F₁ and M₁ plants

genotype	M I T O S I S			M E I O S I S		
	number of plants	normal karyotype	abnormal karyotype	number of plants	only bivalents	occasional quadrivalent
GP X S138	26	26	0	6	6	0
-500rads	27	24	3	10	7	3
-1000rads	17	10	7	3	1	2
-1500rads	4	3	1	-	-	-
-2000rads	1	0	1	-	-	-

- quantitative characters in the second generation

i. height

The analysis of variance in height is summarised in Table 3 (see also Appendix A1). For each parent, any 'within families' variation must have been environmental in origin since individuals were genetically identical. So the results for both parents could be pooled to estimate the environmental mean square for each character. Each within families mean square was tested against its environmental mean square to confirm that there were genetic differences among individuals for each trait. In the case of variation in height, a significant genetic component was identified for all treatments.

Since both main effects (families, reps) in this analysis were random, the appropriate comparison for the between families mean square (MS) was, where it was significant, the interaction MS. Where it was not significant, the interaction MS provided an estimate of error that could be pooled with the within families MS and used to test the significance of the between family MS.

Using this comparison, significant differences in height between families within treatments were identified in all but one of the M_2 groups. However, the F_2 GPXS138 group also tested significantly.

The differences between treatments are displayed in Figures 6 and 7 (where the percentage of plants falling into each phenotypic class is presented), in Figure 8 (where family variances are plotted according to treatment), and in the following table (where means and standard errors of the means for height are given). Group 1 refers to plants derived from GPXS138 crosses, while Group 2 plants are derived from S138xGP crosses.

Table 3: Analysis of variance - height

	within families				between families		
	\bar{x}	MS	df	P	MS	df	P
GP	57.33	26.281	106	-	291.518	2	
S138	64.80	54.982	30	-	52.456	2	
GP X S138	68.26	86.409	336	***	592.778	9	***
-500rads	68.62	92.920	680	***	209.106	24	***
-1000rads	66.92	86.222	372	***	300.201	11	***
-1500rads	67.08	125.510	27	***	-	-	-
S138 X GP	68.93	78.311	318	***	65.344	9	
-500rads	68.52	94.990	545	***	404.636	17	
-1000rads	67.28	95.126	207	***	430.548	6	***

- = non-applicable
 = non significant
 * = $P < 0.05$
 ** = $P < 0.01$
 *** = $P < 0.005$

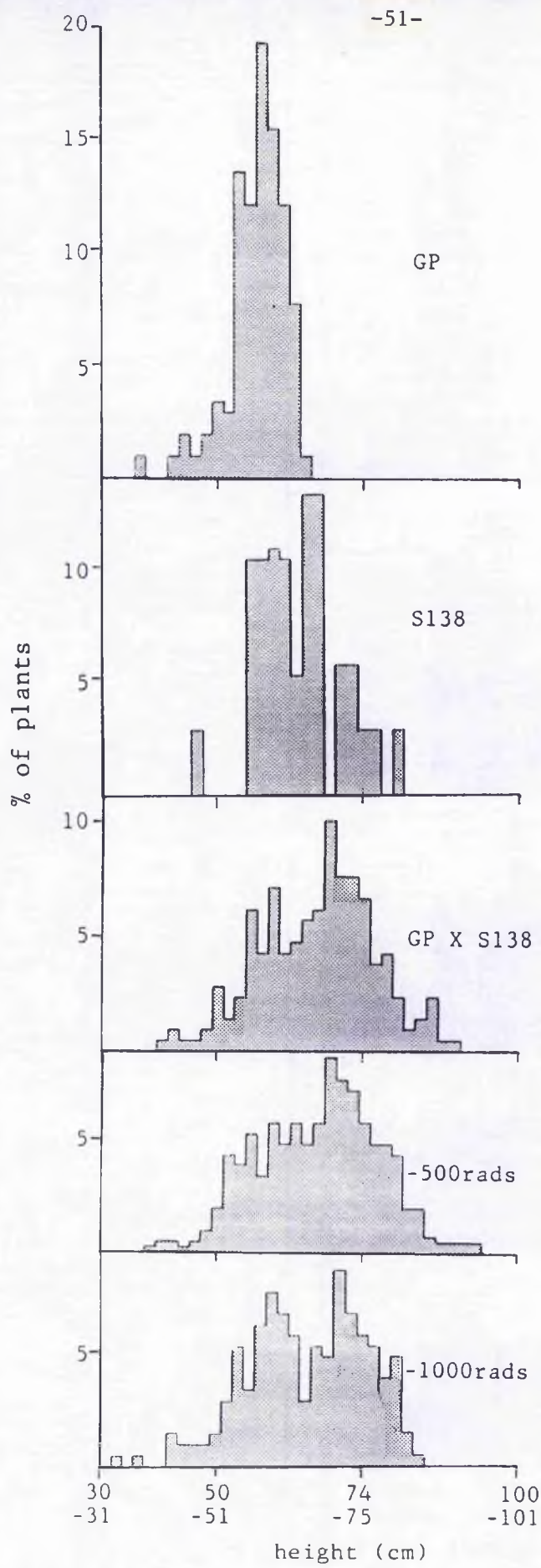


Figure 6: Height distribution in the second generation - GP X S138

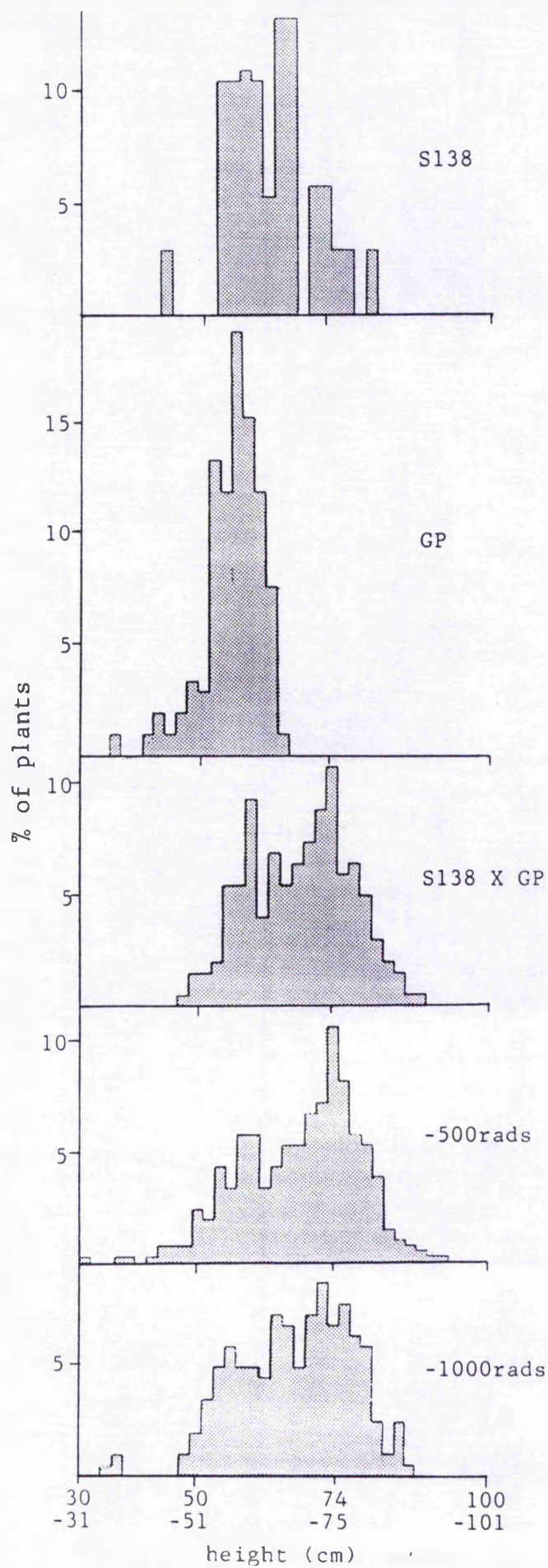


Figure 7: Height distribution in the second generation - S138 X GP

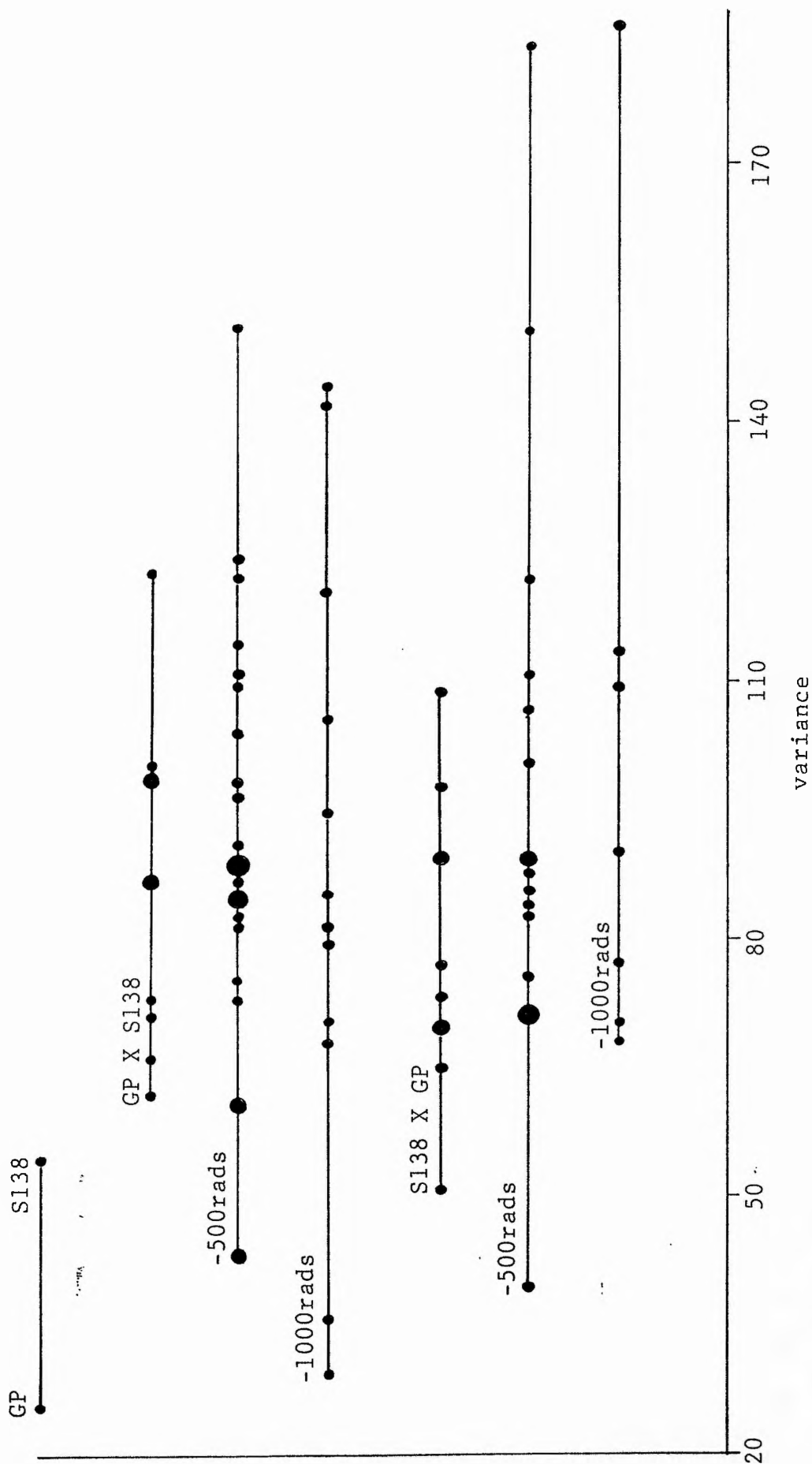


Figure 8: Height variability within second generation families

1 ● 2 ● 3 ● 4 ● 5 ●
number of families

Group 1			Group 2		
F ₂	GPxS138	68.26±0.49	F ₂	S138xGP	68.93±0.48
M ₂	-500	68.62±0.36	M ₂	-500	68.52±0.40
	-1000	66.92±0.47		-1000	67.28±0.66
	-1500	67.08±2.08*			
GP		57.33±0.48	S138		64.80±1.24

*These results are from only one family.

GP was shorter than S138 which was shorter than the F₂. The F₂ means are indistinguishable from one another, as are the reciprocal M₂s at each dose. The data are not inconsistent with a maternal trend of decreasing height with increasing dose. That said, at 500 rads the means remain equivalent to the F₂ means, and at the highest doses they have not fallen as low as the means for either parent.

The frequency distributions shown in Figures 6 and 7 are similar in the irradiated and control crosses. The range of phenotypes was slightly larger in the M₂, both upper and lower limits of the F₂ being extended.

The variability within families was generally greater in the irradiated crosses (Table 3), although the distribution of variances (Figure 8) shows some M₂ families were less variable than the least variable F₂ family and others were considerably more variable than the most variable F₂ family.

ii. tiller number

The analysis of variance in tiller number is summarised in Table 4 (see also Appendix A1). There were significant genetic differences within families for all treatments. Between family differences were significant in one M₂ group and, again surprisingly, in the GPXS138 F₂.

Table 4: Analysis of variance - tiller number

	within families				between families		
	\bar{x}	MS	df	P	MS	df	P
GP	5.42	8.609	106	-	25.589	2	
S138	4.49	11.746	30	-	7.867	2	
GP X S138	5.87	17.668	336	***	44.758	9	**
-500rads	6.36	14.170	680	***	36.325	24	
-1000rads	7.19	26.687	372	***	51.574	11	*
-1500rads	6.08	23.654	27	***	-	-	-
S138 X GP	6.17	17.631	318	***	30.313	9	
-500rads	6.32	22.285	545	***	24.765	17	
-1000rads	6.57	23.156	207	***	10.133	6	

- = non-applicable
 = non significant
 * = $P < 0.05$
 ** = $P < 0.01$
 *** = $P < 0.005$

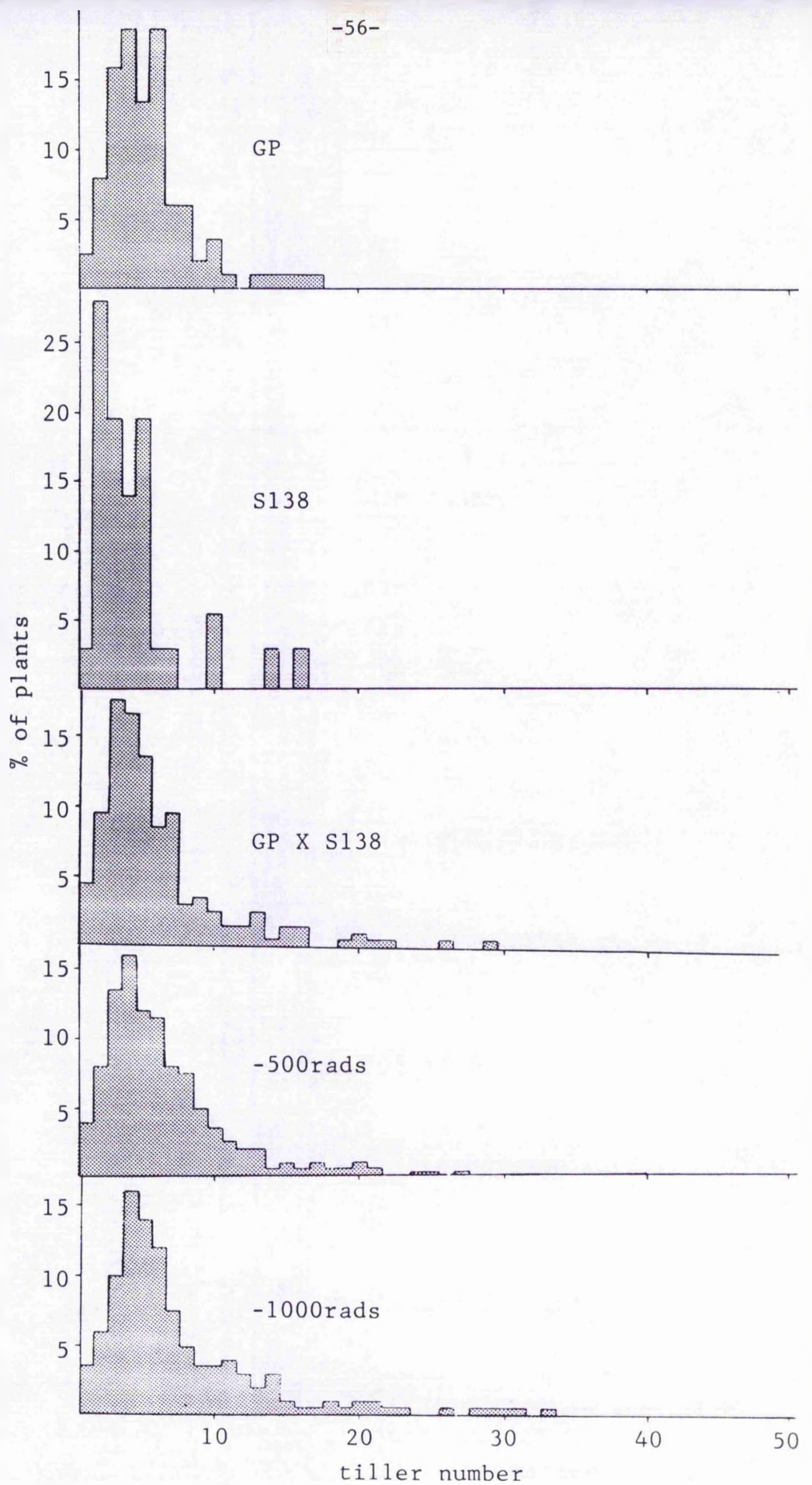


Figure 9: Tiller number distribution in the second generation - GP X S138

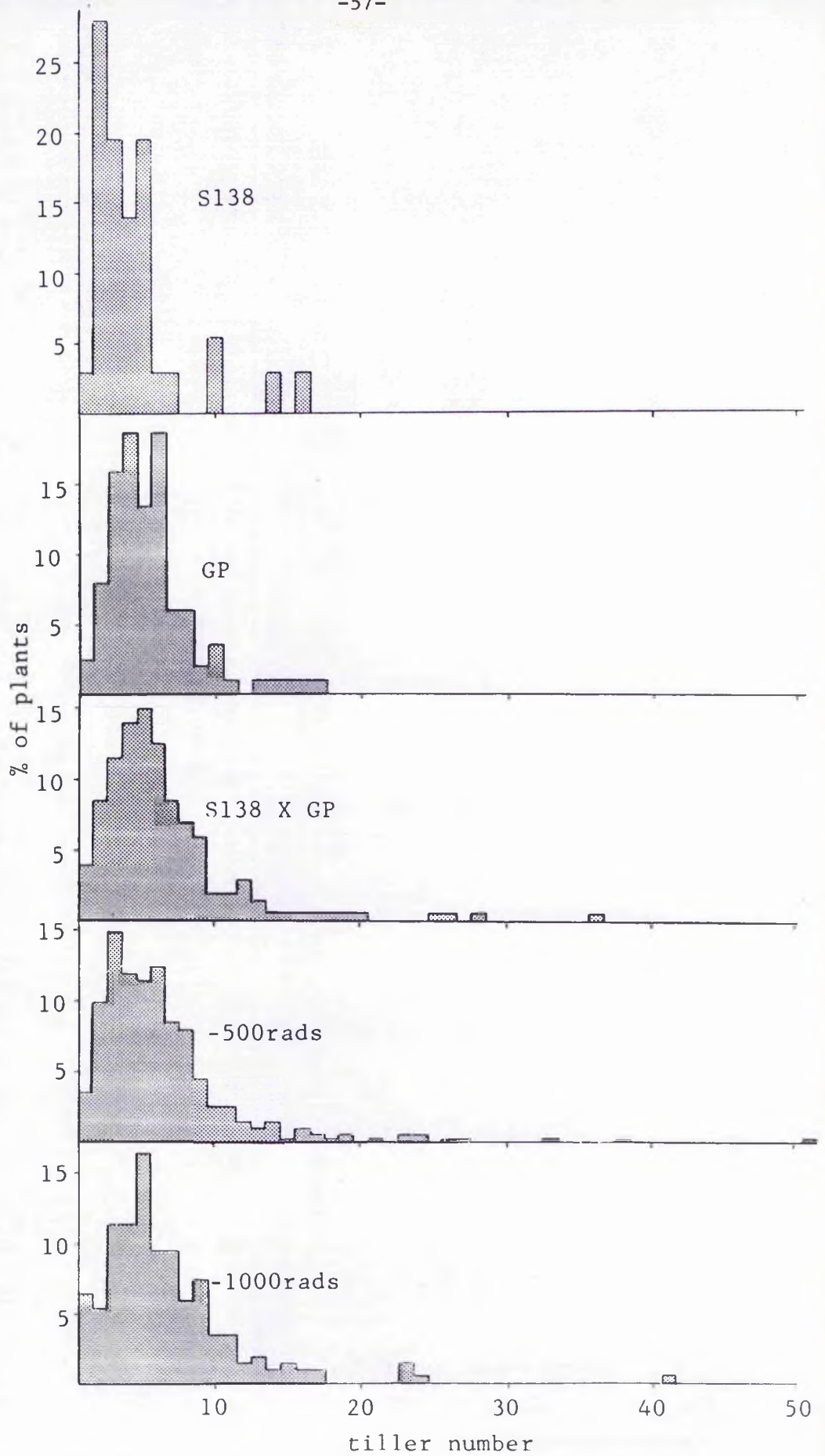


Figure 10: Tiller number distribution in the second generation - S138 X GP

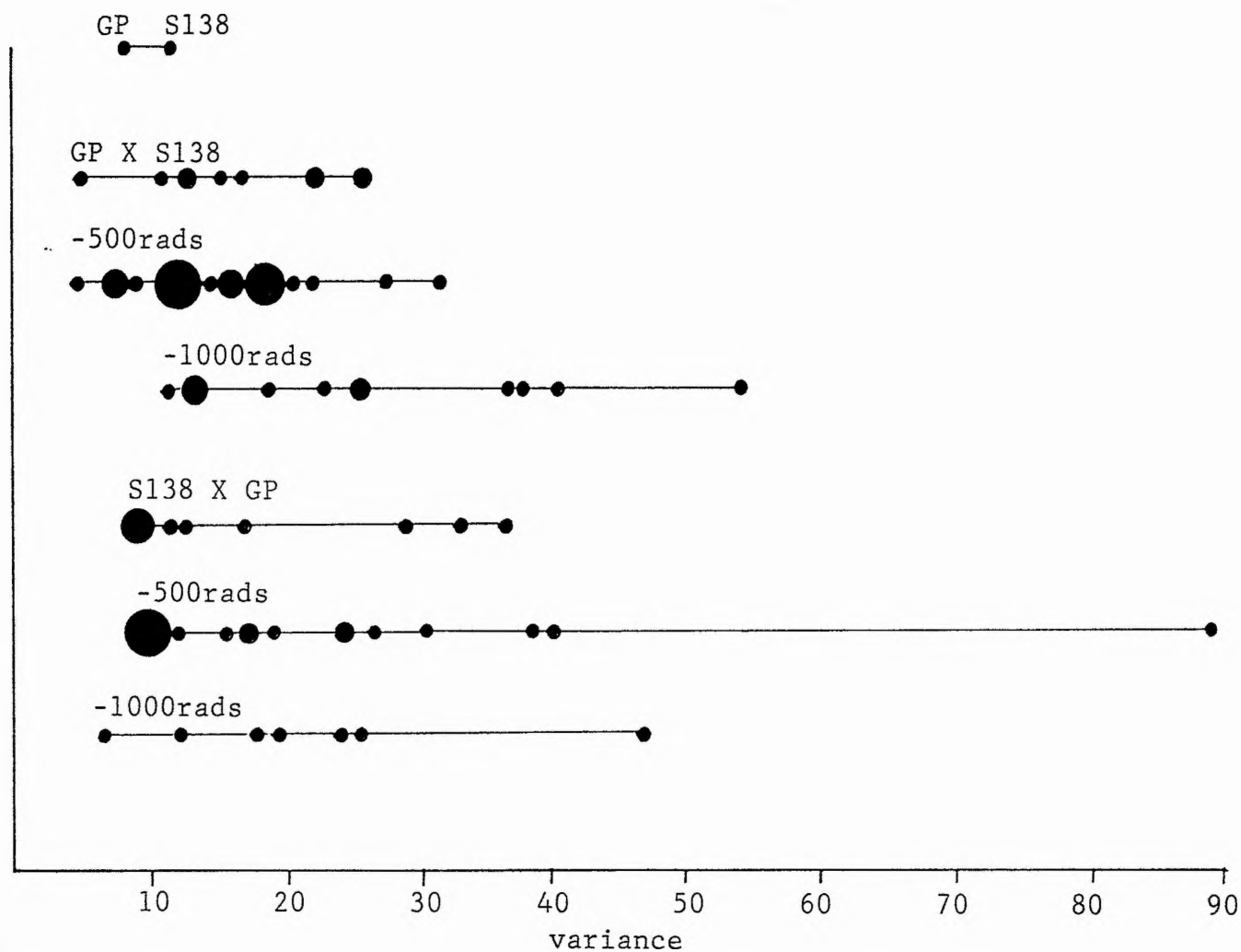


Figure 11: Tiller number variability within second generation families

1 ● 2 ● 3 ● 4 ● 5 ●
number of families

The means and standard errors of the means are presented below:-

Group 1		Group 2	
F ₂ GPxS138	5.87 \pm 0.22	F ₂ S138xGP	6.17 \pm 0.23
M ₂ -500	6.36 \pm 0.14	M ₂ -500	6.32 \pm 0.20
-1000	7.19 \pm 0.26	-1000	6.57 \pm 0.33
-1500	6.08 \pm 0.90		
GP	5.42 \pm 0.28	S138	4.49 \pm 0.57

S138 had fewer tillers than GP which was statistically indistinguishable from the F₂. In group 1, the M₂ actually produced more fertile tillers per plant than did the F₂ or the maternal parent, GP. While in Group 2, the number of tillers produced by plants derived from irradiated crosses was not significantly different from the control number. Neither of these results is consistent with a maternal trend. No change would have been expected in the first group. Whereas in the second, a decrease in tiller number would have been consistent with a maternal trend.

As with height, the frequency distributions (Figures 9 and 10) of the F₂ and the M₂ were very similar. In both groups of crosses, the highest number of fertile tillers per plant was recorded for an M₂ plant.

The variability within most M₂ families fell within the range displayed by their F₂ counterparts (Figure 11). The most variable families did not necessarily result from the highest dose treatments.

iii. ear length

The analysis of variance in ear length (Table 5, see also Appendix A1) reveals significant genetic variation within families for all treatments. While there were significant differences between families within two of the M₂ treatments, the F₂ GPXS138 again tested significantly. The means and standard errors of the means follow:

Table 5: Analysis of variance - ear length

	within families				between families		
	\bar{x}	MS	df	P	MS	df	P
GP	7.78	0.714	106	-	1.620	2	
Sl38	6.19	1.191	30	-	0.041	2	
GP X Sl38	7.30	1.495	336	***	10.699	9	***
-500rads	7.57	1.582	680	***	3.021	24	***
-1000rads	7.45	1.498	372	***	1.718	11	
-1500rads	7.67	1.491	27	*	-	-	-
Sl38 X GP	7.54	1.281	318	***	0.618	9	
-500rads	7.30	1.393	545	***	4.439	17	***
-1000rads	7.46	1.178	207	***	1.832	6	

- = non-applicable
 = non-significant
 * = P < 0.05
 ** = P < 0.01
 *** = P < 0.005

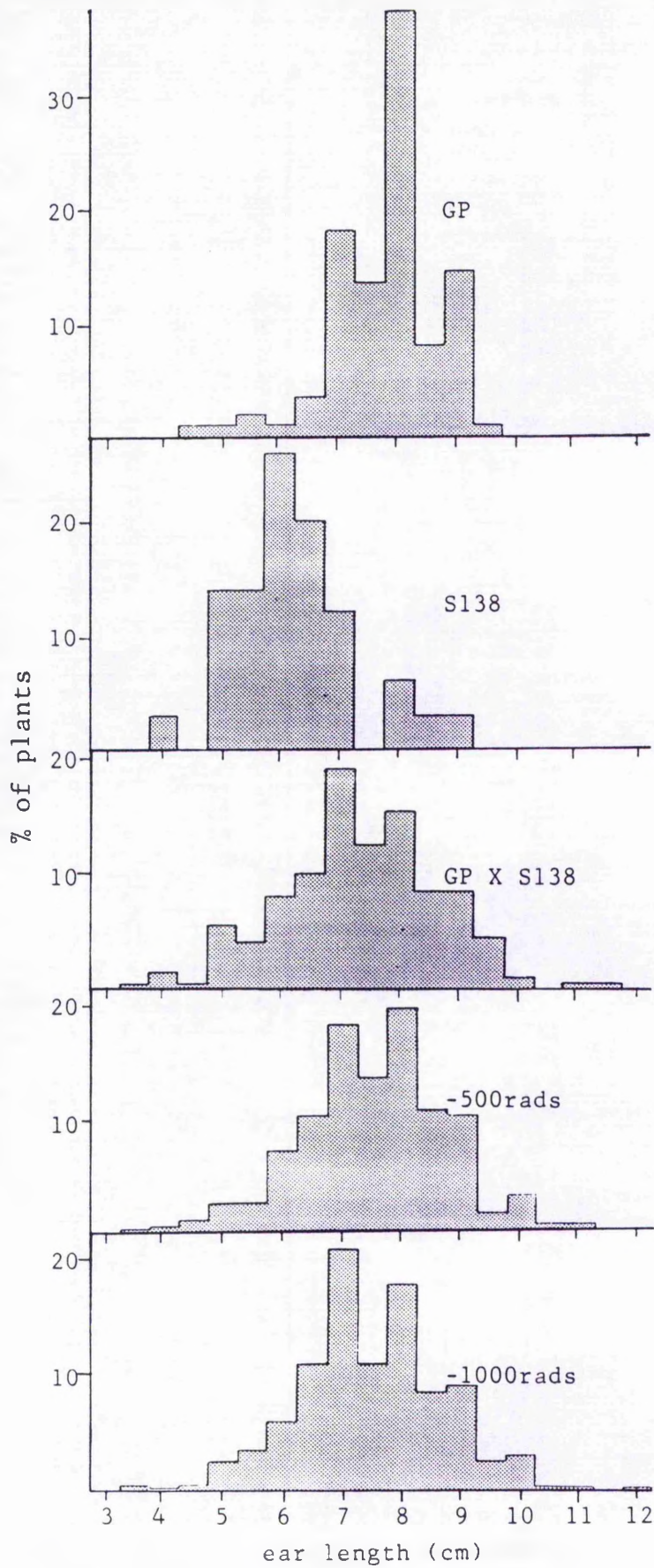


Figure 12: Ear length distribution in the second generation - GP X S138

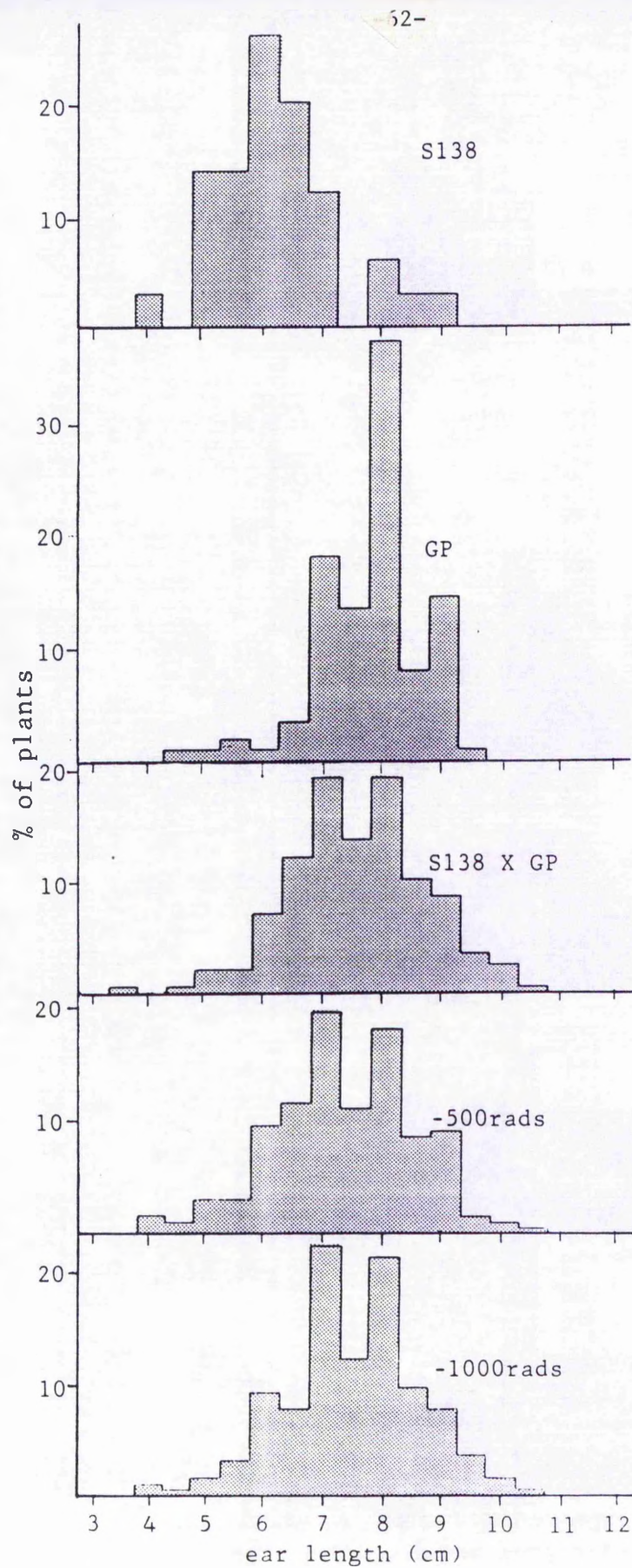


Figure 13: Ear length distribution in the second generation - S138 X GP

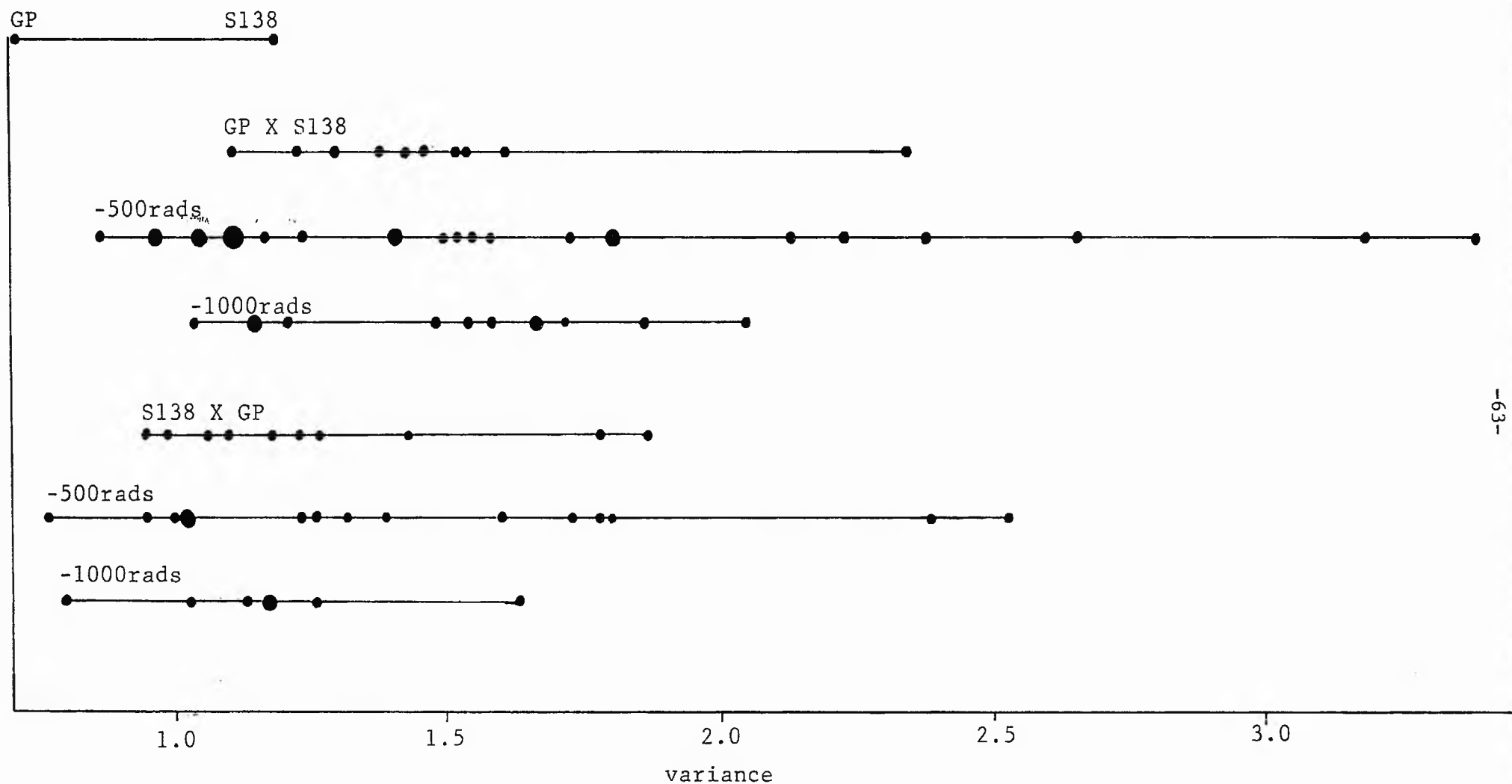


Figure 14: Ear length variability within second generation families

1 ● 2 ● 3 ● 4 ● 5 ●
number of families

Group 1		Group 2	
F_2 GPxS138	7.30 ± 0.06	F_2 S138xGP	7.54 ± 0.06
M_2 -500	7.57 ± 0.05	M_2 -500	7.30 ± 0.05
-1000	7.45 ± 0.06	-1000	7.46 ± 0.07
-1500	7.67 ± 0.23		
GP	7.78 ± 0.08	S138	6.19 ± 0.18

GP had longer ears than S138, both F_2 means being intermediate for this character. In Group 1, M_2 means were larger than that of the unirradiated control, although a trend with dose was not readily apparent. Even so, at the highest dose of 1500rads the mean was statistically indistinguishable from that of the original maternal parent, GP. In Group 2, M_2 means were less than or equal to that of the F_2 , but never as low as the S138 mean. Despite the absence of dose-dependent trends, shifts that did occur were towards the maternal.

Again the frequency distribution of the F_2 and M_2 [Figures 12 and 13] are not dramatically different. Both the least and the most variable families resulted at a dose of 500rads [Figure 14]; the 1000rad treatments produced families more like the unirradiated controls in the variability they displayed.

iv. awn length

A summary of the analysis of variance in awn length is presented in Table 6 (see also Appendix A1). While there was significant genetic variation within families, no significant differences between families within treatments were observed. Surprisingly, the 'between families' component of the genetically uniform S138 was just significant at the 5% level.

Table 6: Analysis of variance - awn length

	\bar{x}	within families			between families		
		MS	df	P	MS	df	P
GP	12.39	0.948	97	-	6.133	2	
Sl38	15.39	2.320	30	-	9.928	2	*
<hr/>							
GP X Sl38	14.69	6.272	287	***	21.133	9	
-500rads	14.83	6.448	615	***	18.252	24	
-1000rads	15.26	6.396	323	***	35.230	11	
-1500rads	16.56	5.141	24	***	-	-	-
<hr/>							
Sl38 X GP	15.14	4.865	275	***	20.967	9	
-500rads	14.82	5.815	477	***	20.182	17	
-1000rads	15.09	5.754	173	***	15.809	6	

- = non-applicable
 = non-significant
 * = $P < 0.05$
 ** = $P < 0.01$
 *** = $P < 0.005$

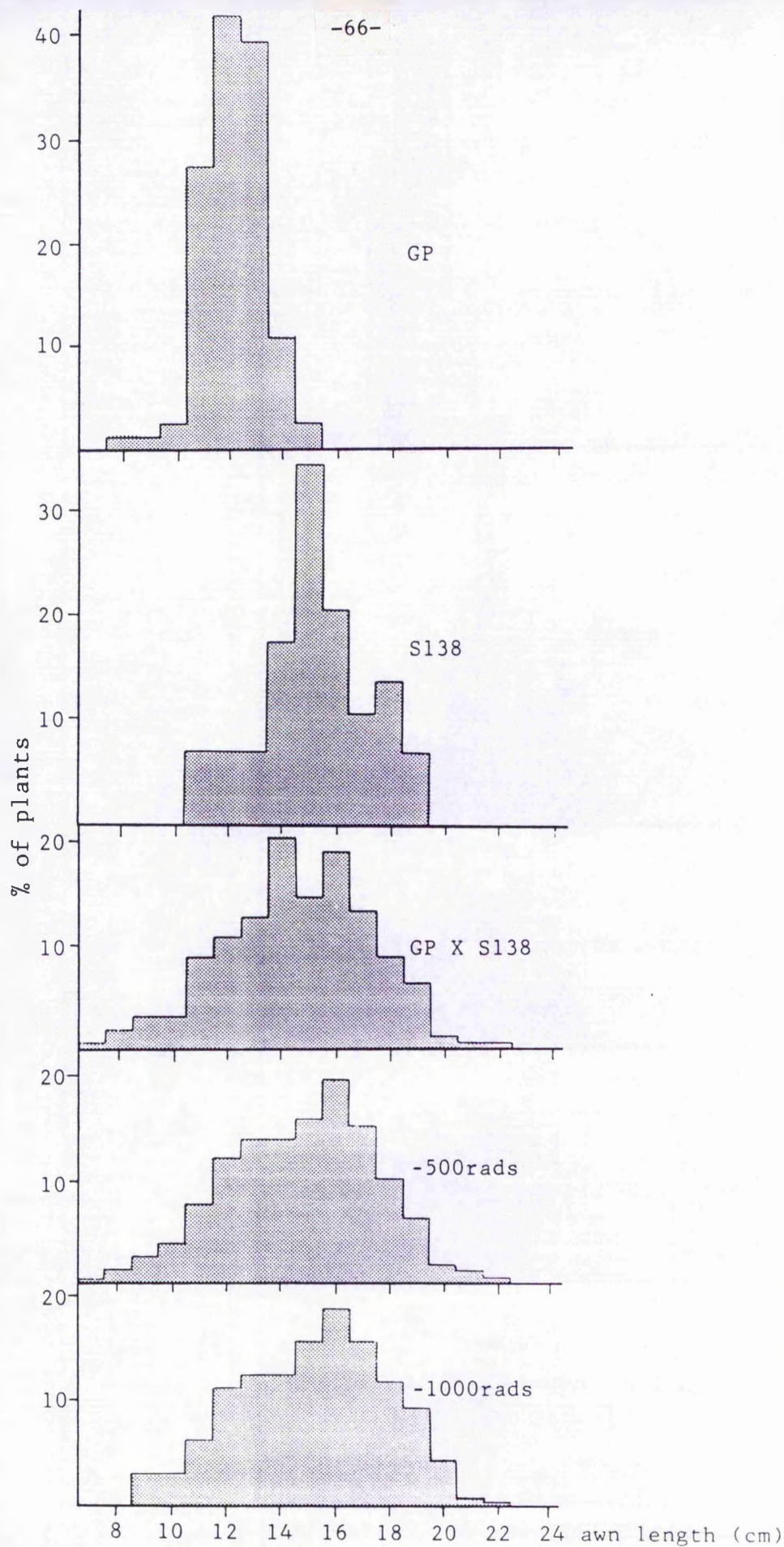


Figure 15: Awn length distribution in the second generation - GP X S138

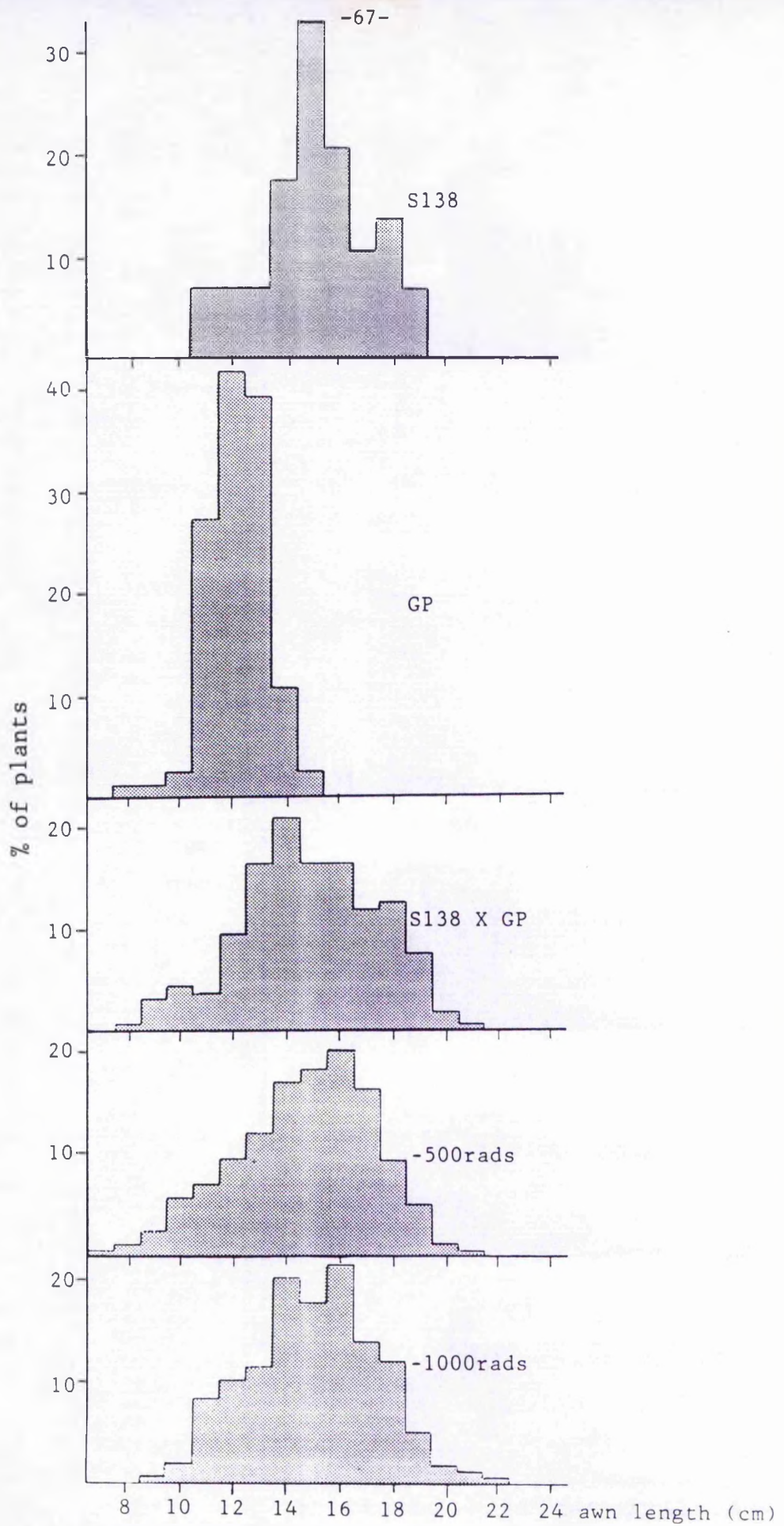


Figure 16: Awn length distribution in the second generation - S138 X GP

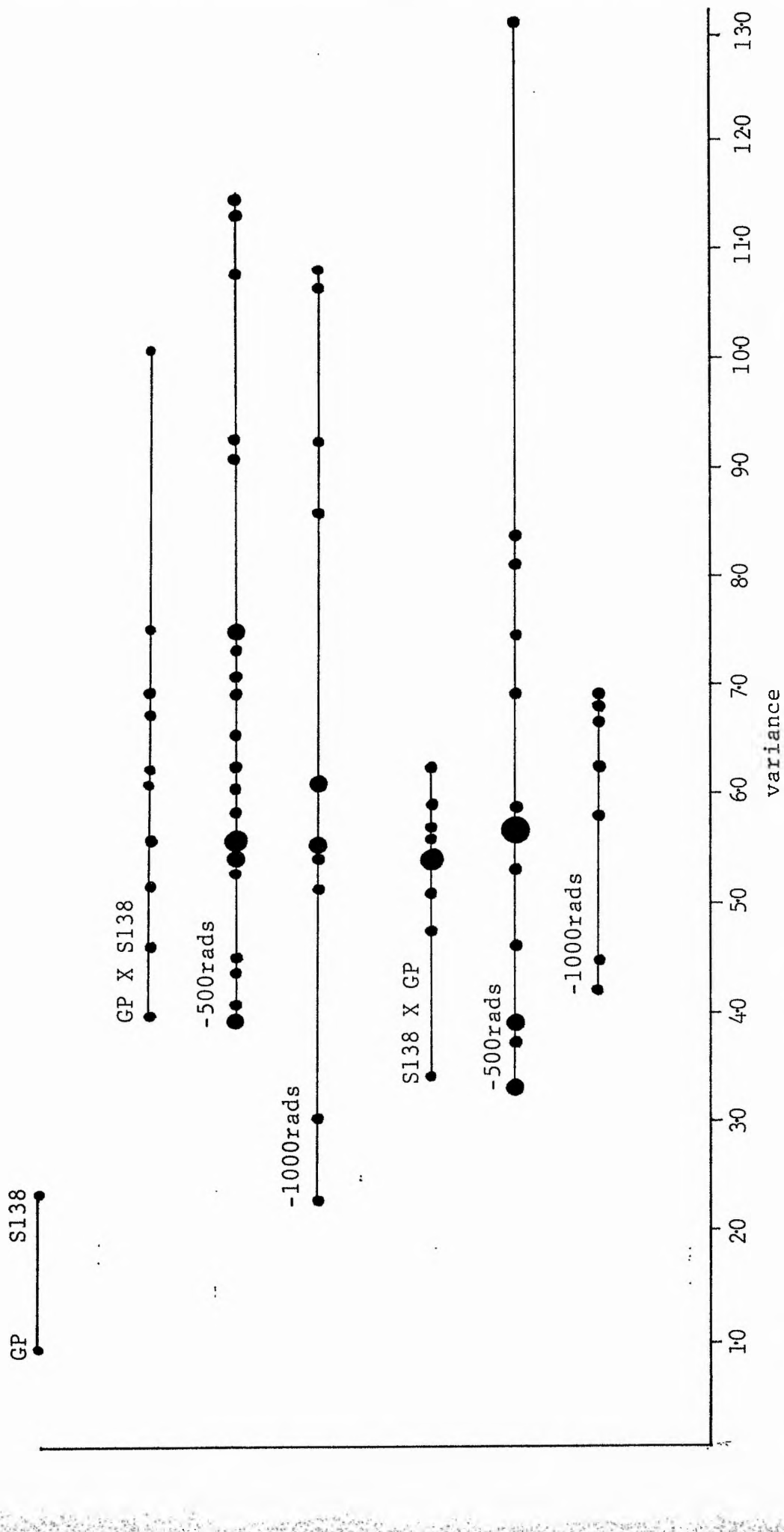


Figure 17: Awn length variability within second generation families

1 ● 2 ● 3 ● 4 ● 5 ●
number of families

The differences between treatments can be seen in the means and standard errors of the means set out below:

Group 1		Group 2	
F ₂ GPxS138	14.69±0.14	F ₂ S138xGP	15.14±0.13
M ₂ -500	14.83±0.10	M ₂ -500	14.82±0.11
-1000	15.26±0.14	-1000	15.09±0.18
-1500	16.56±0.44		
GP	12.39±0.10	S138	15.39±0.25

GP had shorter awns than S138 which was statistically indistinguishable from either F₂. In Group 1 there was a trend away from the maternal, awn length increasing with increasing dose. Whereas in Group 2, little difference existed between the F₂, M₂ and maternal means.

Again, the frequency distributions of the F₂ and M₂ [Figures 15 and 16] are similar. While the variability displayed by most M₂ families was within the range exhibited by the F₂, there were examples of both more and less variable families [Figure 17].

v. green tiller number

The results for green tiller number are shown in Table 7 and in Figures 18, 19 and 20.

Apart from in two treatment groups, phenotypic variation for this character was consistent with that due to environmental differences alone (Table 7). Indeed, both GP and S138 were intermediate in variance (Figure 20). The frequency distributions (Figures 18 and 19) are similar for all groups, with approximately 75% of plants having 0, 1 or 2 green tillers each.

Table 7: Analysis of variance - green tiller number

	\bar{x}	within families			between families		
		MS	df	P	MS	df	P
GP	2.41	3.038	106	-	95.981	2	
S138	1.47	3.577	30	-	1.827	2	
GP X S138	1.34	3.655	336		9.360	9	
-500rads	1.50	3.575	680		6.785	24	
-1000rads	1.78	4.241	372	*	20.633	11	
-1500rads	2.73	5.820	27	*	-	-	-
S138 X GP	1.30	3.263	318		4.079	9	
-500rads	1.42	3.890	545		7.598	17	
-1000rads	1.57	3.819	207		13.776	6	

- = non-applicable
 = non-significant
 * = $P < 0.05$
 ** = $P < 0.01$
 *** = $P < 0.005$

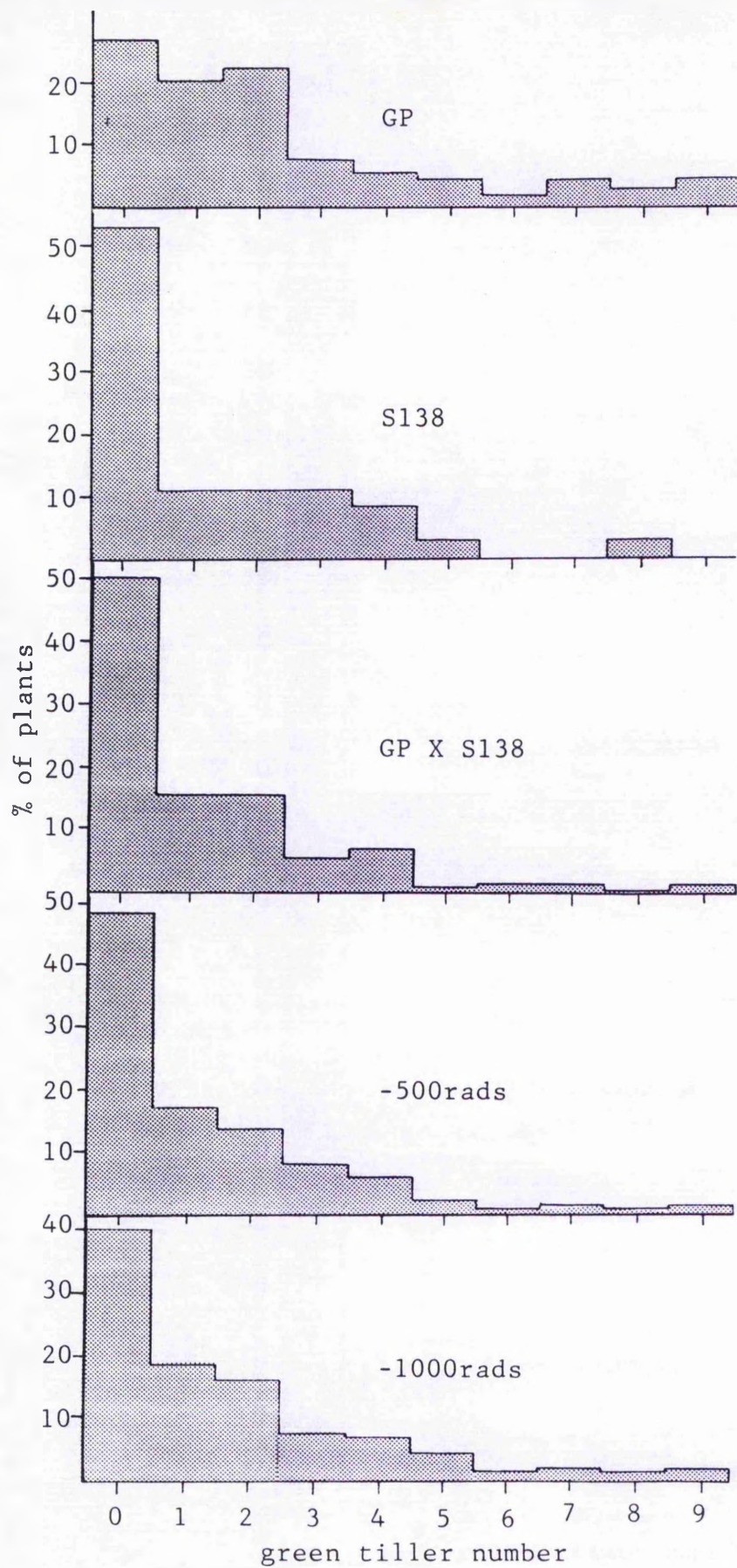


Figure 18: Green tiller number distribution
in the second generation - GP X S138

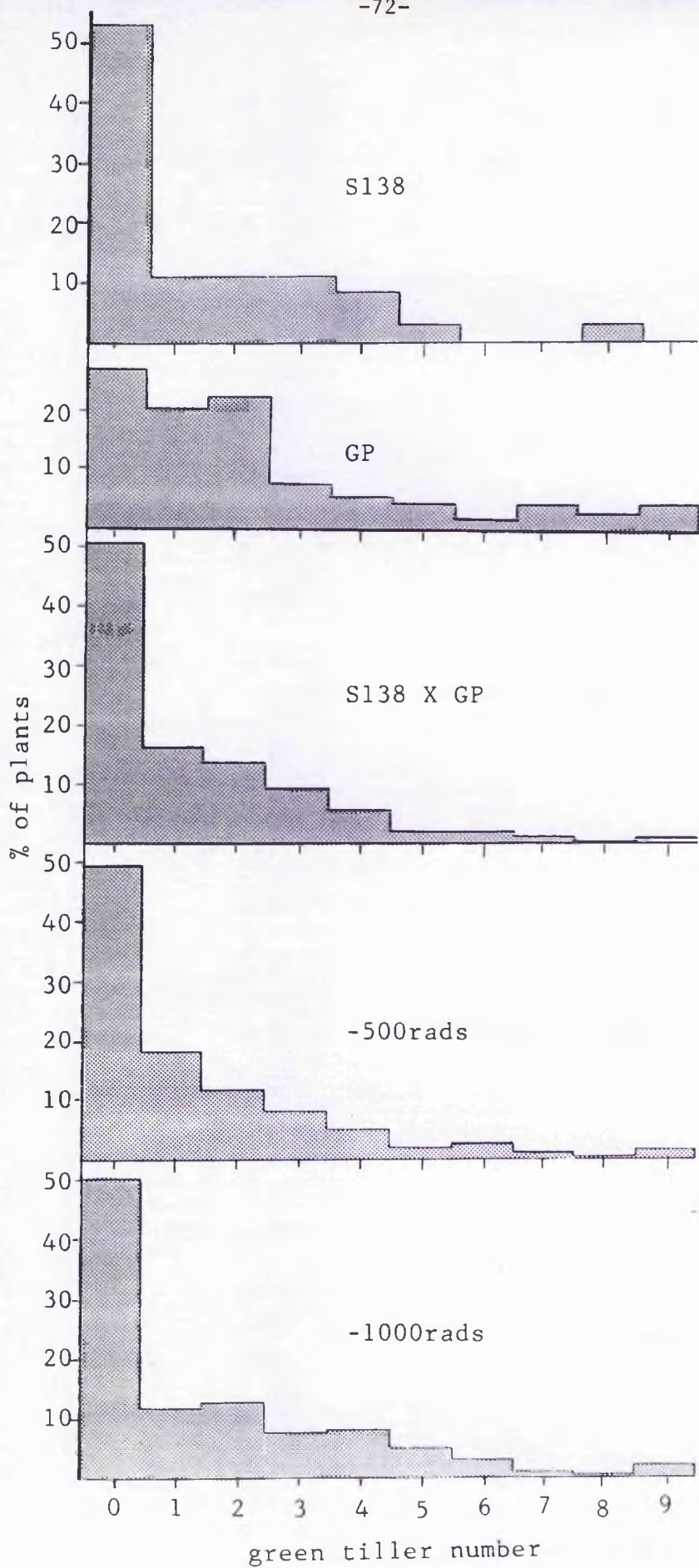


Figure 19: Green tiller number distribution in the second generation - S138 X GP

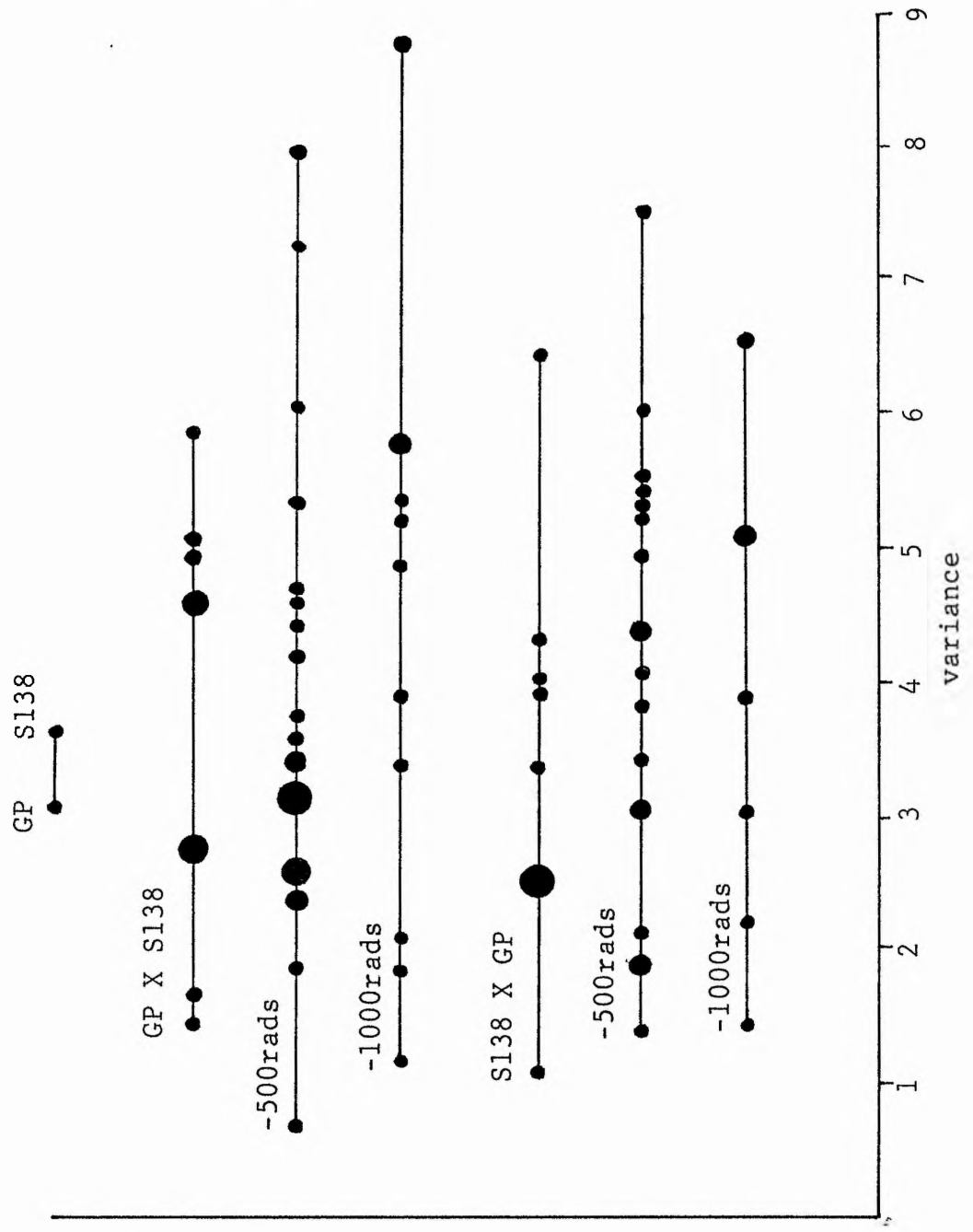


Figure 20: Green tiller number variability within second generation families

1 • 2 • 3 • 4 • 5
number of families

- qualitative characters in the second generation

Initially, the data for each gene scored were considered individually (see Appendix A2). If the ratio of dominant to recessive phenotypes was consistent for families within a treatment, then (sampling error apart) the ratio for each family should agree with the overall ratio for that treatment. This hypothesis was tested by means of heterogeneity χ^2 analyses, the results of which are presented in Table 8.

While F_2 families would be expected to be homogeneous with regard to phenotypic frequencies, the non-targeted nature of the radiation treatment might result in significant differences between M_2 families derived from the same dose. In fact, for fragile vs non-fragile stem and for black vs white lemma and pericarp, even F_2 families were inconsistent. For both genes this is probably a reflection of the difficulties in scoring mentioned earlier.

To recap, as plants were harvested it was difficult to tell if they were fragile because they were homozygous for 'fs', or whether their fragility was a consequence of ageing. And seed colour wasn't black or white either! A number of apparently 'grey' seeded individuals were designated black as it was assumed the seeds would have turned this colour had plants been harvested later. This probably resulted in an over-estimate of the number of plants carrying the 'B' allele. This would also explain the 4.48:1 and 6.51:1 ratios obtained for the F_2 s (rather than the expected 3:1). In view of the uncertainty over data for these two markers, neither was considered further.

Having examined the homogeneity of treatment groups, the phenotypic frequencies of each M_2 family (and, where families were consistent, of each treatment) were compared with those of the F_2 using the χ^2 goodness of fit test (see Appendix A3).

	Fs:fs	P	R:r	P	S:s	P	B:b	P	Ert:ert	P
GP	I:0	-	1:0	-	1:0	-	0:1	-	1:11.4	-
SL38	0:1	-	0:1	-	0:1	-	1:0	-	1:0	-
GPxSL38	2.62:1	0.0009 ***	3.26:1	0.4484	3.11:1	0.5564	4.48:1	0.0279 *	3.72:1	0.6444
-500rads	2.43:1	0.1106	2.97:1	0.5930	3.61:1	0.3172	4.03:1	0.0863	3.19:1	0.3466
-1000rads	1.66:1	0.0000 ***	4.79:1	0.0043 ***	4.62:1	0.0078 **	4.05:1	0.3147	2.36:1	0.0062 **
-1500rads	[8.76:1]	-	[3.14:1]	-	[2.63:1]	-	[3.14:1]	-	[3.14:1]	-
SL38xGP	2.67:1	0.2023	2.52:1	0.0730	2.89:1	0.9639	6.51:1	0.0359 *	2.92:1	0.5271
-500rads	2.75:1	0.0495 *	4.29:1	0.1863	3.56:1	0.6734	4.41:1	0.0514	3.22:1	0.5976
-1000rads	2.75:1	0.8784	3.70:1	0.2210	4.68:1	0.9329	3.91:1	0.5987	3.02:1	0.6740

[] = results from only one family

Table 8: Major gene frequencies in the F₂ and M₂ with the results of heterogeneity chi² tests

When either:-

1. the number of degrees of freedom was 1 and the expected value for a class was less than 5
or
2. the number of degrees of freedom was greater than 1, but there were classes with expected values of less than 1

a correction for continuity was made.

In the first case, the appropriate adjustment was brought about by reducing each of the deviations (observed-expected) by one half. In the second, smaller classes were combined so that the expected value in all new classes was at least 1, and the number of degrees of freedom was adjusted accordingly.

i rough:smooth awns (R : r)

Apart from families derived from the GPxS138 cross at 1000 rads, the ratio of rough to smooth awned plants was consistent for families within treatments (see Table 8). Since the F_2 s were not significantly different, data from both were pooled before being compared with each M_2 . The segregation ratio for each treatment is given below together with the direction of shift from the F_2 and, where applicable, the significance of the shift.

	GP:S138
pooled F_2	2.87:1
GXS-500	2.97:1 maternal N.S.
-1000	4.79:1 maternal
SXG-500	4.29:1 paternal ***
-1000	3.70:1 paternal N.S.

Each M_2 had a higher proportion of dominant phenotypes than the F_2 . But, where this excess could be tested, it was not always significant.

When families were considered separately (see Appendix A3), only 1 out of the 25 GXS-500 families was significantly different from the F_2 , the excess being of paternal types. In the same cross at 1000 rads, 3 out of 12 families had significantly more maternal types, 1 of these having no paternal phenotypes at all. In the reciprocal irradiated cross, the 2 significantly different families both resulted from the 500 rad treatment, and both had a higher proportion of paternal phenotypes than did the F_2 .

ii long:short rachilla hairs (S : s)

Again the χ^2 test for heterogeneity was only significant in the case of the GPXS138 cross at 1000 rads. And, once more, each irradiated treatment produced an excess of dominant phenotypes regardless of the direction of the cross:-

	GP:S138
pooled F_2	3.01:1
GXS-500	3.61:1 maternal *
-1000	4.62:1 maternal
SXG-500	3.56:1 paternal N.S
-1000	4.68:1 paternal *

Two of the GXS-500 rad families were significantly different from the F_2 at the 5% level; one had an excess of paternal phenotypes and the other more maternal types. At 1000 rads, the two significantly different families both had a larger proportion of maternal types. None of the reciprocal M_2 families were significantly different from the F_2 , despite a significant excess of paternal types when data was pooled for the 1000 rad treatment.

iii tall:dwarf (Ert : ert)

Once more, the GPXS138-1000 families were the only ones that were inconsistent. But unlike rough:smooth awn and long:short rachilla hairs, there was an excess of recessive phenotypes in the treated groups. Where it could be tested, this excess was not significant:-

	S138:GP
pooled F_2	3.30:1
GXS-500	3.19:1 maternal N.S
-1000	2.36:1 maternal
SXG-500	3.22:1 paternal N.S
-1000	3.02:1 paternal N.S

1 out of 25 families resulting from the GPXS138 cross at 500 rads had a significant excess of maternal phenotypes when compared to the F_2 . At 1000 rads, 3 families were significantly different. 2 of these had more, and 1 less, of the recessive maternal types. In the reciprocal cross only 1 out of the 25 families was significantly different. Again the excess was of recessive (but this time paternal) phenotypes.

- linkage study: qualitative characters

The genetic markers focused on in this study were all located on barley chromosome 7; GPert lies on the short arm, while awn type and rachilla hair type lie on the long arm.

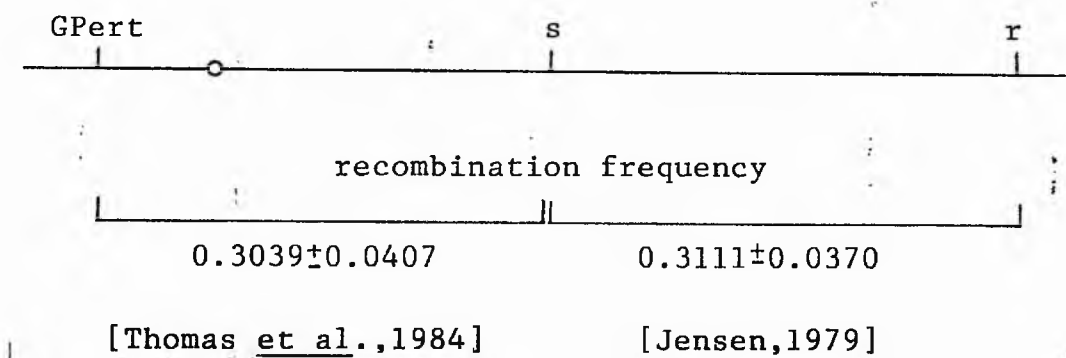


Figure 21: Genetic map of barley chromosome 7

The F_2 segregation ratio obtained for each combination of genes was tested against the 9:3:3:1 ratio expected in the case of independent inheritance:-

	χ^2	df	P
r & s	124.25	3	***
s & GPert	27.73	3	***
r & GPert	3.73	3	N.S.

Given that 'GPert' and 'r' are fairly distantly located on the chromosome (i.e. more than 50 centimorgans apart), it is not unexpected that crossing over between them occurred often enough to suggest they were assorting independently. For the two more closely associated combinations, linkage was confirmed by highly significant departures from the 9:3:3:1 ratio. F_2 segregation ratios were then compared with those obtained from the M_2 .

The phenotypic frequencies of M_2 families that departed significantly from the F_2 are given in Tables 9, 10 and 11, together with sources of variation. (This is intended as only a rough guide to the relative contribution of each variation source, the number of plants assessed for each gene and for combinations differed slightly. The remainder χ^2 is what was left once the χ^2 for each gene involved was subtracted from the χ^2 for the combination in question.)

In many M_2 families, much of the departure from the F_2 in linked combinations could be traced back to disturbed ratios for the individual genes involved. There were cases, however, where the ratios of individual genes were consistent with those of the F_2 . So in these instances, significant differences must have been recombinational in origin.

Considering, for example, the combination of rough/smooth awns and long/short rachilla hairs (Table 9). The original cross involved homozygous parents, one doubly dominant, the other doubly recessive. The only phenotypic class containing no recombinants (excluding double cross-overs) was 'rs', while most recombinants would have been 'RS'.

	PHENOTYPIC FREQUENCY				SOURCE OF VARIATION (χ^2)		
	RS	Rs	rS	rs	R:r	S:s	Remainder*
pooled F_2	.631	.117	.106	.146	-	-	-
<hr/>							
GXS-500 (21)	.647 H	.088 L	.235 H	.039 L	0.01	2.50	6.13
(pooled)	.663 H	.085 L	.120 H	.132 L	1.94	4.06	3.56
-1000 (1)	.879 H	.000 L	.030 L	.091 L	2.57	3.63	3.37
(4)	.833 H	.028 L	.111 H	.028 L	2.68	6.24	-0.68
(6)	.867 H	.133 L	.000 L	.000 L	9.14	1.59	-0.43
(12)	.914 H	.043 L	.043 L	.000 L	4.47	4.17	-0.45
<hr/>							
SXG-500 (8)	.844 H	.125 L	.031 L	.000 L	7.47	2.03	-0.80
(pooled)	.697 H	.114 L	.087 L	.102 L	14.12	2.76	-3.37
-1000 (7)	.765 H	.059 L	.088 L	.088 L	1.18	1.92	10.78
(pooled)	.690 H	.097 L	.134 H	.079 L	2.33	6.26	1.57

L = lower than F_2 , H = higher than F_2 , *see text

Table 9: M_2 families with phenotypic frequencies significantly different from those of the F_2

	PHENOTYPIC FREQUENCY				SOURCE OF VARIATION (χ^2)		
	RERT	Rert	rErt	rert	R:r	Ert:ert	Remainder*
pooled F_2	.546	.201	.203	.050	-	-	-
<hr/>							
GXS-500 (16)	.482 L	.074 L	.407 H	.037 L	4.89	0.82	2.32
-1000 (2)	.472 L	.444 H	.028 L	.056 H	4.88	14.06	-2.55
(4)	.805 H	.056 L	.139 L	.000 L	2.68	5.67	2.40
(6)	.640 H	.360 H	.000 L	.000 L	9.14	0.75	-0.06
<hr/>							
SXG-500 (8),	.813 H	.156 L	.000 L	.031 L	7.47	0.37	3.36
(pooled)	.608 H	.204 H	.152 L	.036 L	14.12	0.14	-0.89

L = lower than F_2 , H = higher than F_2 , *see text

Table 10: M_2 families with phenotypic frequencies significantly different from those of the F_2

	PHENOTYPIC FREQUENCY				SOURCE OF VARIATION (χ^2)		
	Sert	Sert	sErt	sert	S:s	Ert:ert	Remainder*
pooled F_2	.511	.216	.241	.032	-	-	-
<hr/>							
GXS-500 (2)	.774 H	.129 L	.097 L	.000 L	3.10	1.36	4.23
(pooled)	.563 H	.220 H	.197 L	.020 L	4.06	0.12	8.74
-1000 (2)	.405 L	.405 H	.108 L	.081 H	1.33	14.06	-1.27
(4)	.888 H	.056 L	.056 L	.000 L	6.24	5.67	8.75
<hr/>							
SXG-500	.561 H	.224 H	.199 L	.016 L	2.76	0.04	8.63
(pooled)							
-1000	.592 H	.234 H	.160 L	.014 L	6.26	0.31	4.71
(pooled)							

L = lower than F_2 , H = higher than F_2 , *see text

Table 11: M_2 families with phenotypic frequencies significantly different from those of the F_2

In each significantly different M_2 , the frequency of 'rs' was lower, and the frequency of 'RS' higher, than in the F_2 . This occurred irrespective of which parent had been the irradiated pollen donor and, although in some cases is a reflection of a reduction in the number of recessive alleles, also suggests an increase in the frequency of recombination following radiation treatment.

The cases of awn type, or rachilla hair type, in combination with the erectoides gene are more difficult to interpret. Since both GP and S138 are homozygous dominant for one character and homozygous recessive for the other, each phenotypic class may contain recombinants. Most recombinants would be expected to be phenotypically dominant and for most significantly different M_2 (Tables 10 and 11) there is an excess of this phenotypic class over that of the F_2 . However, this class may also include non-recombinants.

When awn type, rachilla hair type and stature were examined together, the segregation ratios of three M_2 families differed significantly from that of the F_2 (Table 12 - see also Appendix A3). Two of the three were from the GPXS138 cross. In both of these the departure could be traced back to significant disturbances in the individual segregation ratios of 2 of the 3 genes. In one case this resulted in an excess of maternal types (RSert) at the expense of most other classes; in the other the most popular class, RSert, was almost twice as frequent as it had been in the F_2 .

The same explanation could not account for the deviation of the third family, a derivative of the S138XGP cross at 1000 rads. Here individual gene frequencies were consistent with those of the F_2 . Classes that were rare in the F_2 tended to increase in frequency while the two most popular classes decreased. For example, the frequency of rSert (the result of at least 2 cross-overs) rose more than six-fold, and the frequency of RSert (requiring at least 3 cross-overs) rose five-fold. In this family, it would seem that an increased frequency of recombination was responsible for the departure from the F_2 .

PHENOTYPIC FREQUENCY

	RSert	RSert	RSert	rSert	Rsert	rSert	rsert	rsert
pooled F_2	.442	.190	.105	.077	.012	.028	.126	.020
GXS-1000 (2)	.417	.389	.056	.000	.056	.028	.028	.028
(4)	.778	.086	.028	.111	.000	.000	.028	.000
SXG-1000 (4)	.250	.125	.125	.187	.063	.187	.063	.000

Table 12: M_2 families with phenotypic frequencies significantly different from those of the F_2

- linkage study: quantitative characters

As well as the major gene analysis, the relationship between quantitative characters was studied to see what, if any, effect the radiation treatment had had. The investigation focused on three of the characters measured: height, tiller number and ear length. The degree of closeness of the relationship between each pair of variables was measured in a correlation analysis, giving a correlation coefficient (r) for each pair of characters in each family. In addition, a χ^2 test was performed to see if the r 's obtained for a given pair of variables within a treatment were estimates of the same ρ (the population correlation coefficient). Finally, the results from each M_2 family were compared with the combined estimates of r for the F_2 (details of procedures are in Appendix A4).

The distributions of correlation coefficients are shown in Figures 22, 24 and 26, with the results of the χ^2 tests in Table 14. In all cases the F_2 was homogeneous whereas the M_2 was variable. More heterogeneity occurred in the GPXS138 cross than its reciprocal, and at a dose of 500rads rather than 1000rads.

When it came to distribution, the pattern of correlation coefficients appeared more dose- than cross-related. For in each case, by far the widest range occurred at a dose of 500rads. Surprisingly perhaps, the 1000rad groups were more like the F_2 in their distribution.

Generally speaking, each pair of variables was similarly correlated for both Golden Promise and S138 r being low and positive. The exception to the rule was the relationship between height and ear length in the case of GP - where the correlation coefficient was higher at +0.5841. So here approximately 34% of the variability in one character was linked to variability in the other.

		P		
		ht & tn	ht & el	tn & el
F_2	GXS	0.4685	0.2426	0.8580
M_2	GXS-500	0.0032	0.0097	0.0289
		***	***	*
	-1000	0.3456	0.0475	0.3046
			*	
F_2	SXG	0.4778	0.4999	0.5159
M_2	SXG-500	0.0668	0.2587	0.0065

	-1000	0.3212	0.4270	0.8054

Table 14: Results of χ^2 tests for heterogeneity
of the correlation coefficients for each
treatment

While the F_2 distributions tended to centre on the parental values for r , the striking feature of the M_2 was the downward extension of its distribution at 500rads. Although the negative values of r were never bigger than 0.4, it's interesting that in some M_2 families there should be an association between high scores for one character and low scores for another.

When individual M_2 families were compared to the combined F_2 estimate of r , 40% of them were found to differ significantly (see Appendix A4). For 74% of these, significant differences occurred in 1 of the 3 pairs of variables tested; in the remaining 36%, differences occurred in two.

Figures 23, 25 and 27 are scatter diagrams of the parents, the F_2 s and those M_2 families that differed significantly from the controls. In the case of height and tiller number (Figure 23), the F_2 s had low positive correlation coefficients and all but two of the M_2 s had low negative correlations. So the differences between them are not great. Furthermore, when the sample size is small, as in the case of the M_2 families a single point can make a great deal of difference to the correlation coefficient. In any event, there certainly weren't clusters of M_2 individuals with low scores for both characters.

When it came to height and ear length (Figure 25), the F_2 s had relatively high positive correlations. The M_2 s were equally split, half being more significantly positively correlated and half having significantly lower correlation coefficients. Again there were no clusters of M_2 individuals in the low scoring region of the diagram.

The scatter diagrams for tiller number and ear length are presented in Figure 27. Most of the M_2 families that were significantly different to the F_2 had lower correlation coefficients. In the two examples where this wasn't the case, GX5 500(18) and GX5 1000(1), the difference seemed to be due to one or two individuals that were high scoring for both these measures of fertility.

S138 GP
↑ ↓

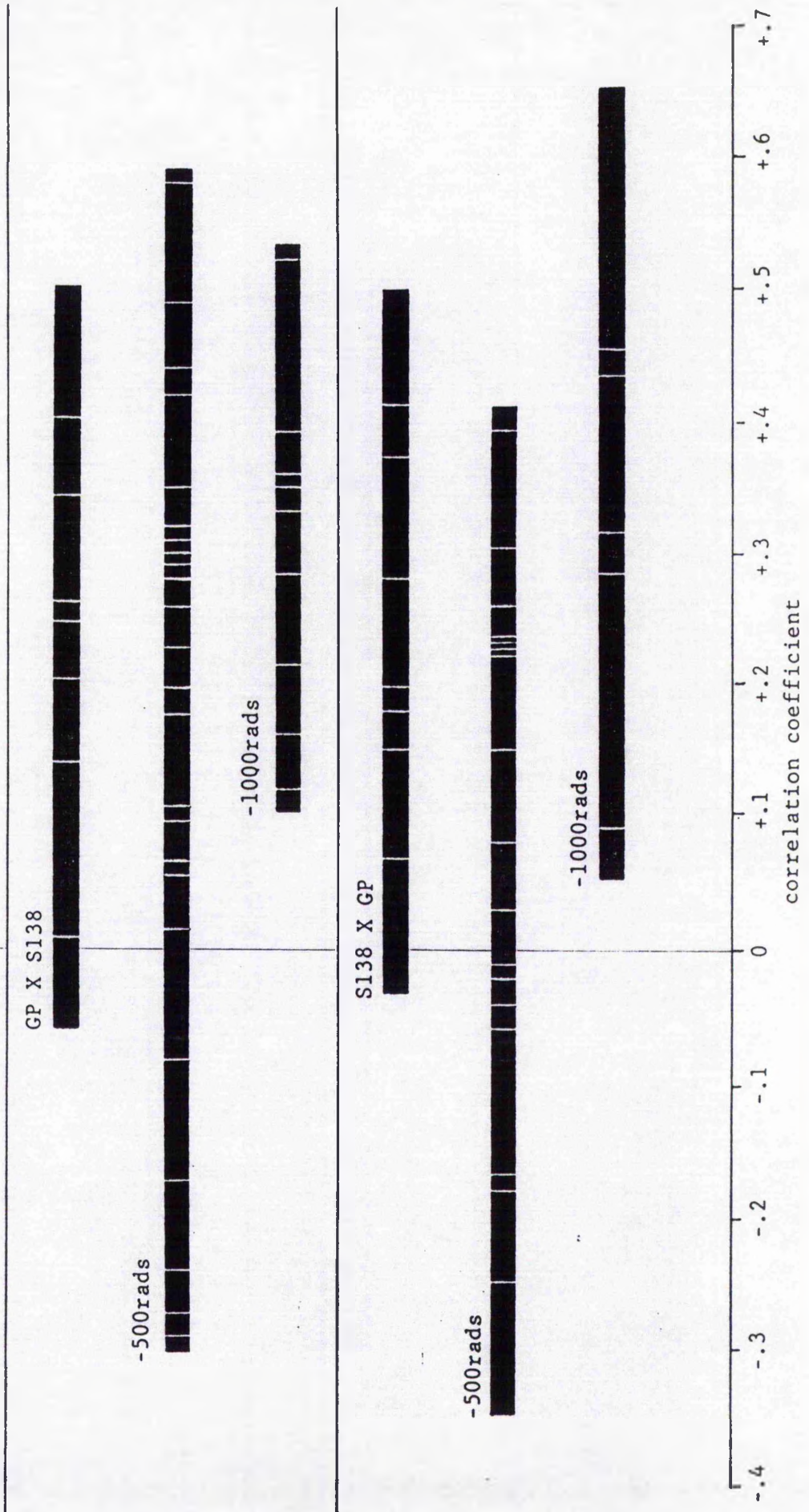


Figure 22: Distribution of 'r' for tiller number and height

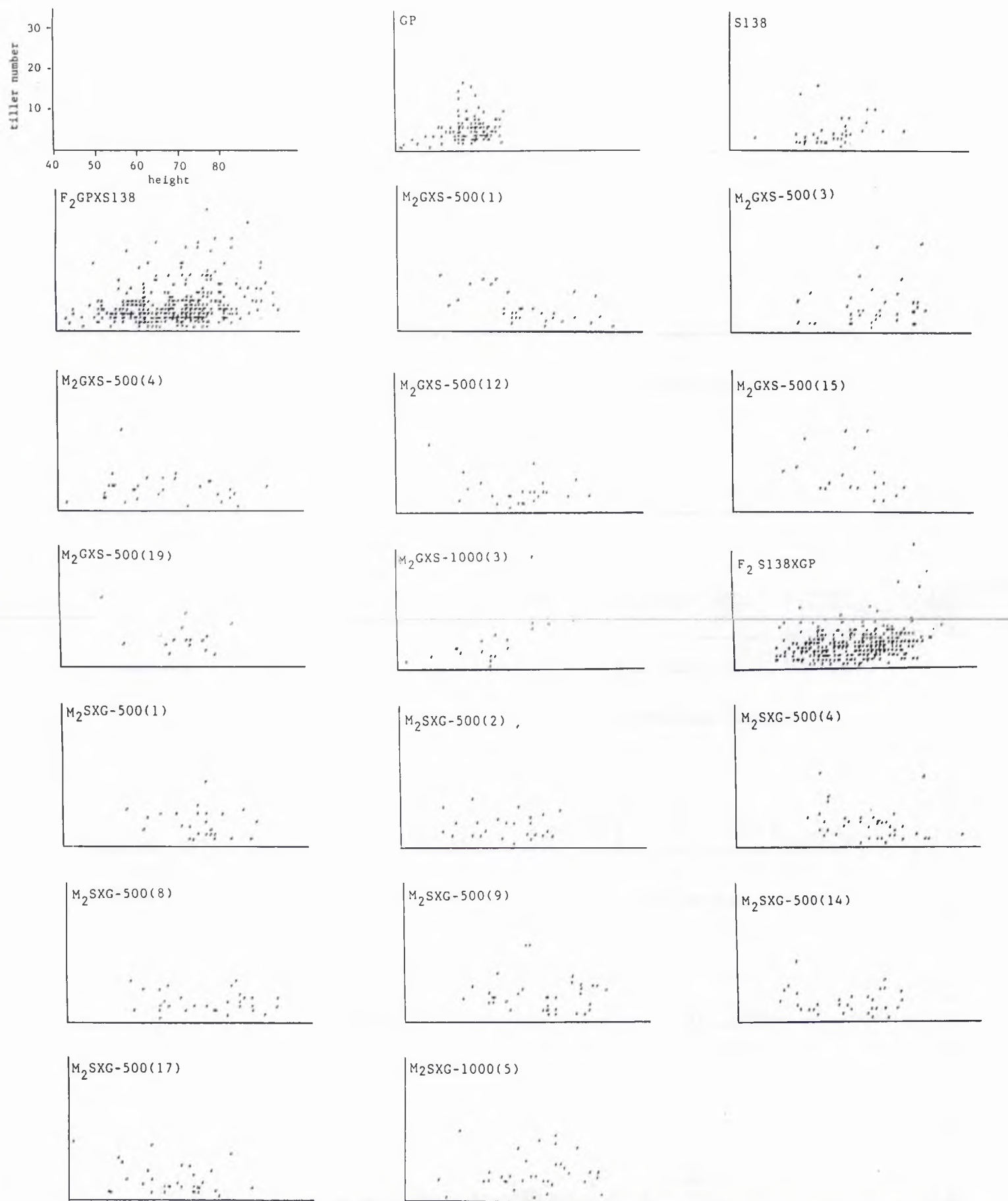


Figure 23: Height/tiller number scatter diagrams for the parents, the F₂s and significantly different M₂ families

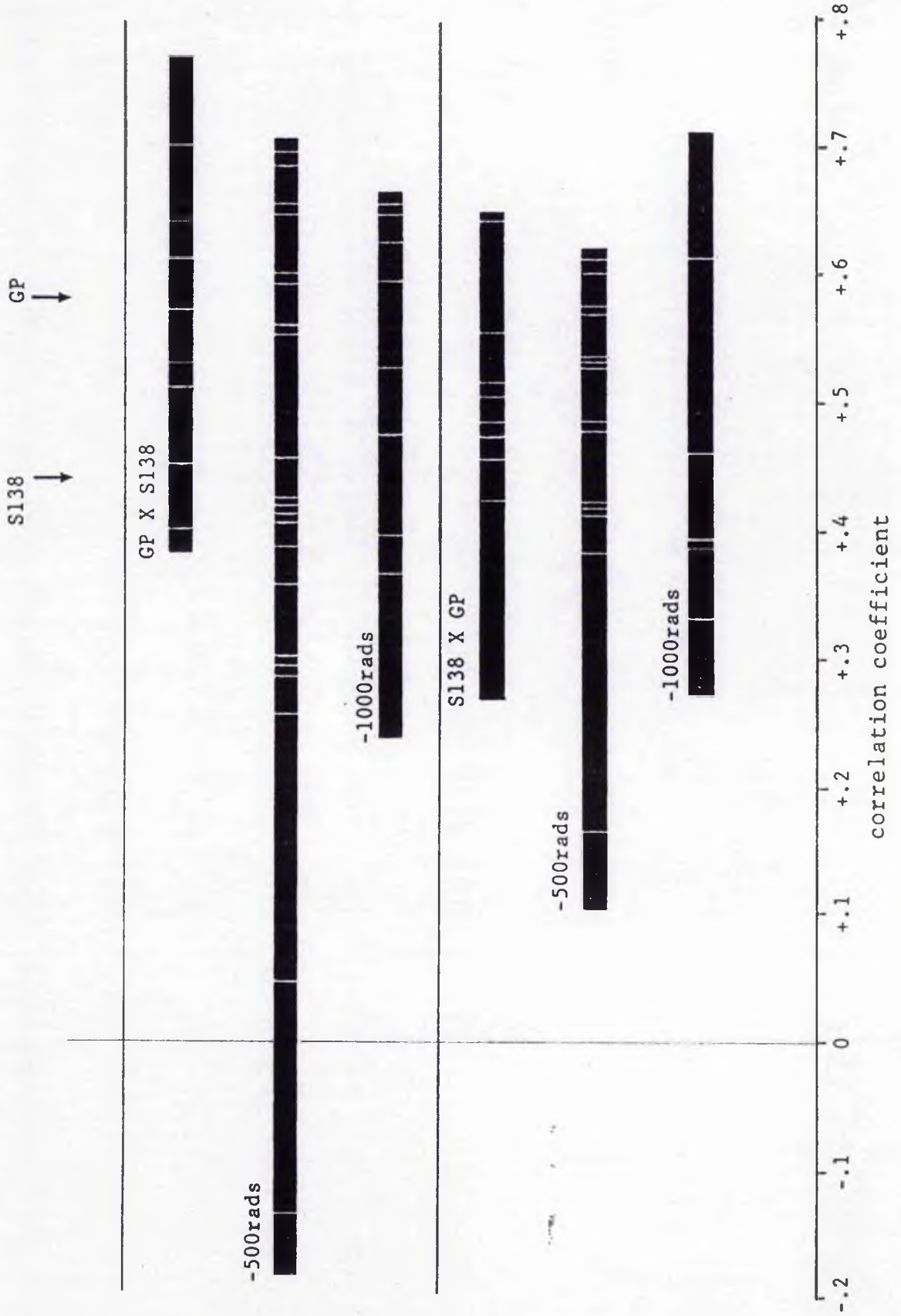


Figure 24: Distribution of 'r' for height and ear length

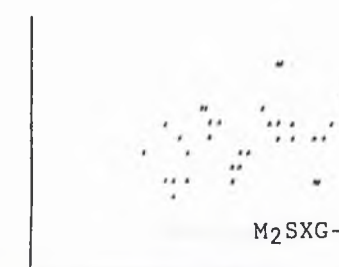
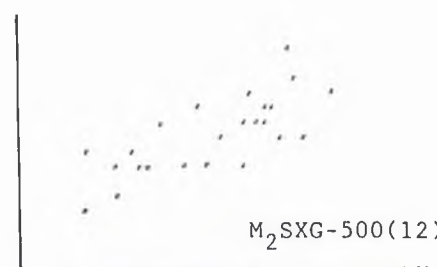
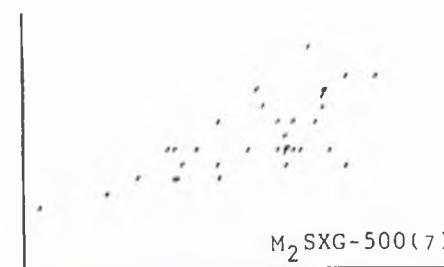
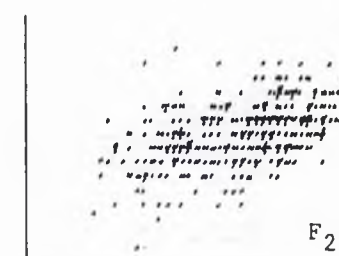
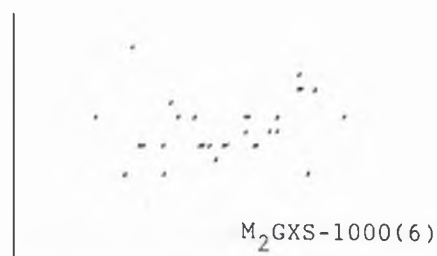
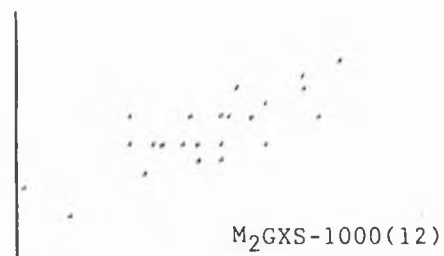
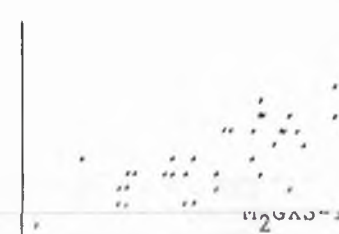
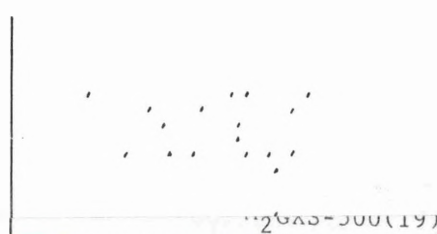
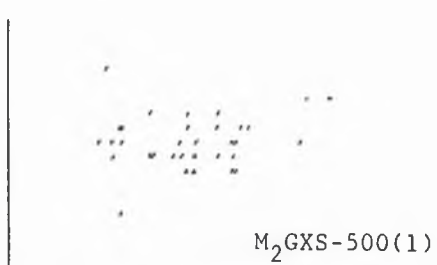
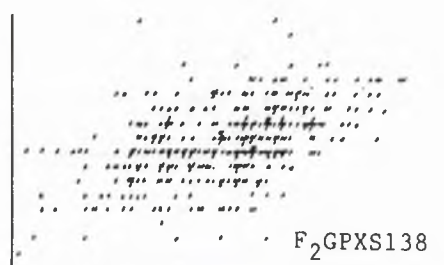
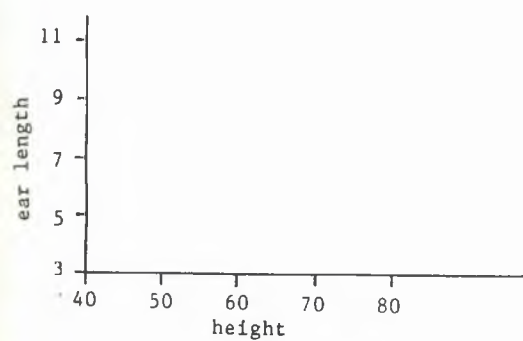


Figure 25: Height/ear length scatter diagrams for the parents, the F₂s and the significantly different M₂ families

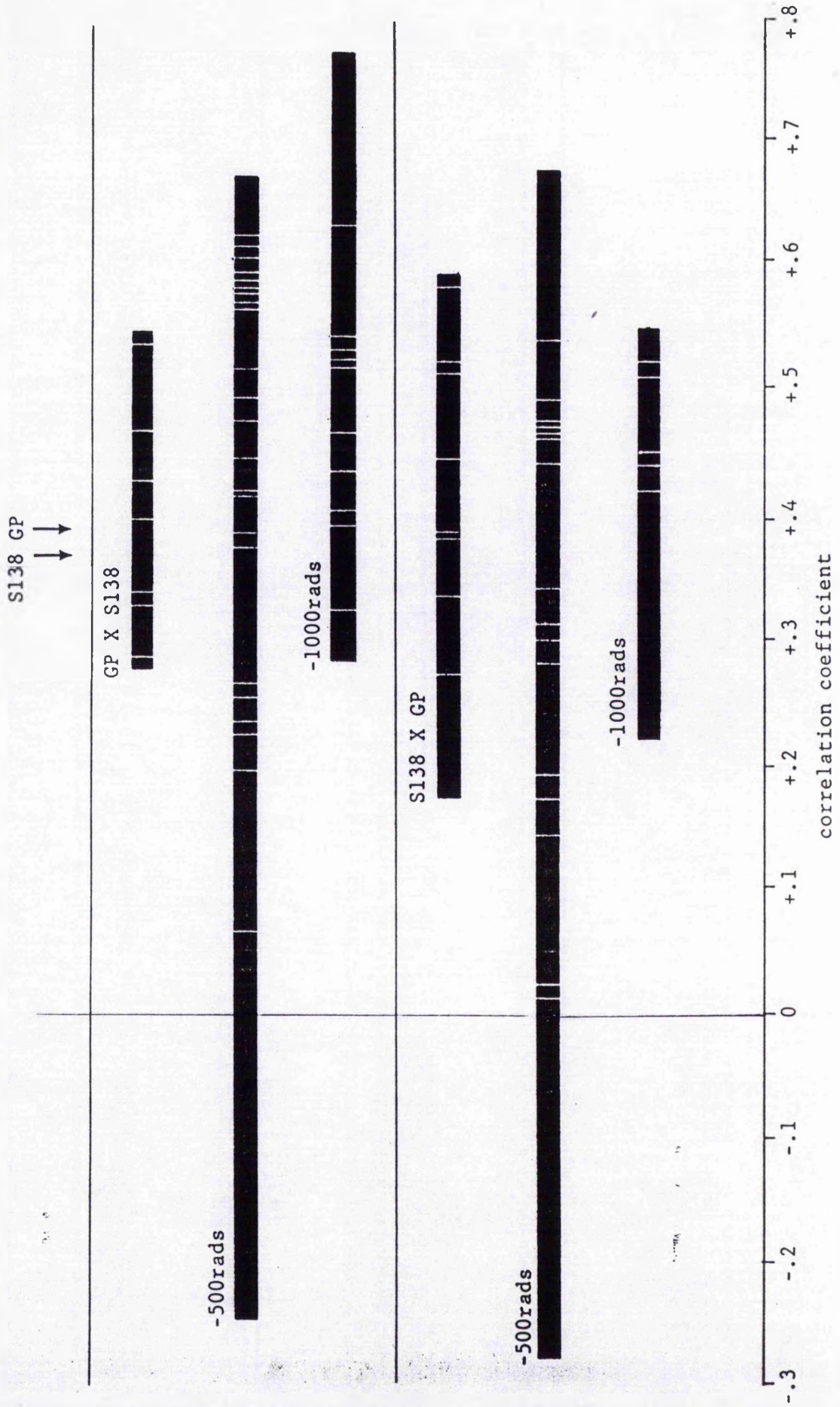


Figure 26: Distribution of 'r' for tiller number and ear length

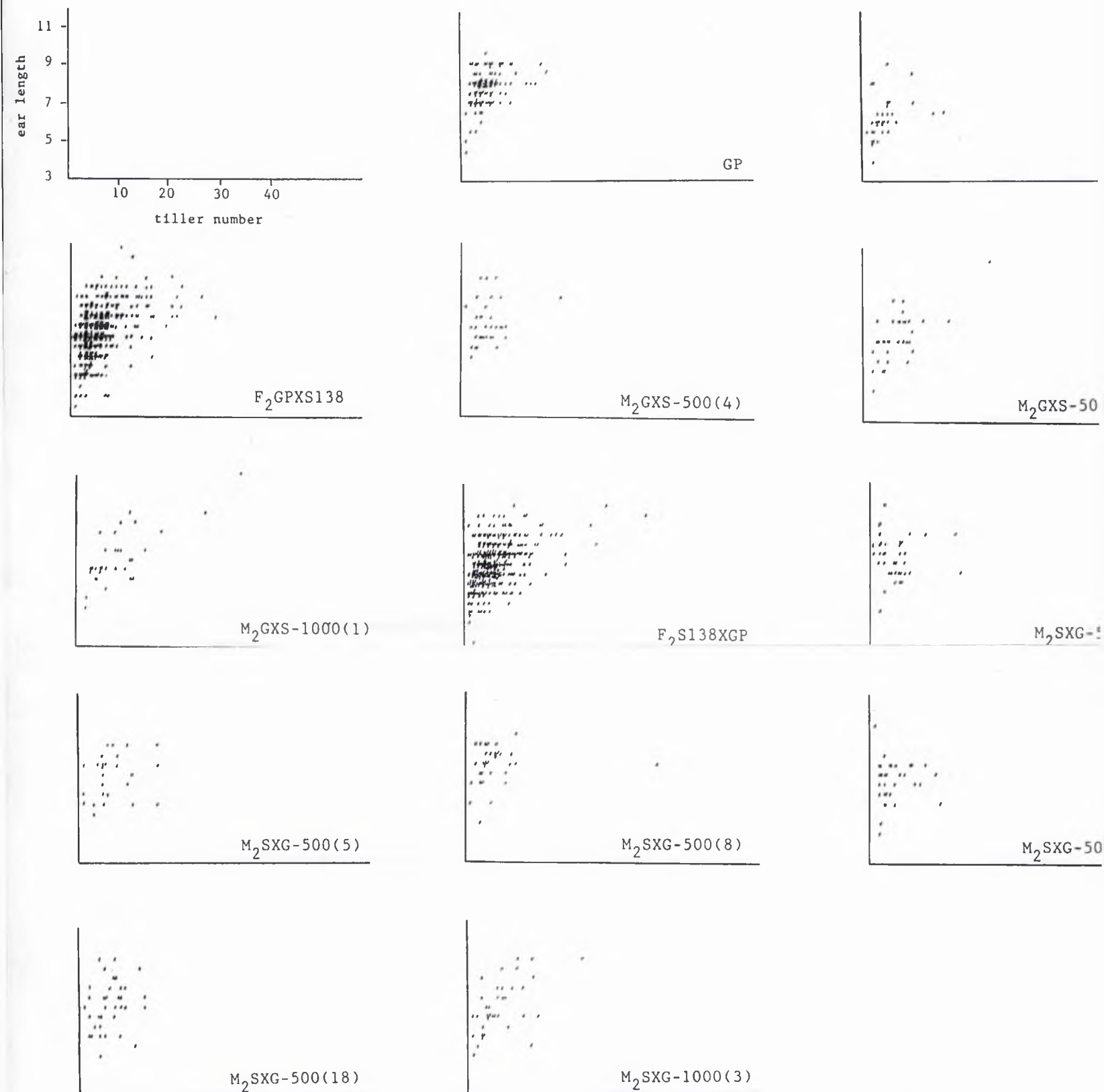
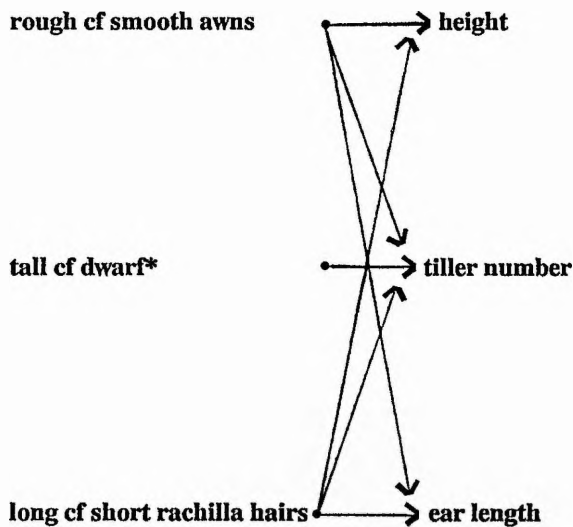


Figure 27: Ear length/tiller number scatter diagrams for the parents, the F₂s and significantly different M₂ families



- linkage study: qualitative and quantitative characters

As well as the analysis of qualitative and that of quantitative traits, the effect of irradiated pollen on linkage between both sorts of characters was investigated. By using t-tests, quantitative scores for dominant phenotypes were compared with those of the recessive phenotypes according to the schedule below:



*Since both height and ear length were used to classify the erectoides gene, tiller number was the only comparison that could be made.

In the case of tiller number (Table 15), dominant phenotypes were to a greater or lesser extent more productive than their recessive counterparts. This was true for each gene considered, although most significant for rachilla hair type, failing to reach significance in the case of awn type. When it came to tall vs dwarf plants, linkage was only significant (and highly so) for GPXS138-1000 rads.

When families within treatments were considered individually, a more variable picture emerged. In order not to confuse environmental and linkage effects because of the small sample size (for a given family all the dominant phenotypes may have been in one rep, the recessive types in the other), data from each rep were considered separately (see Appendix A5).

TILLER NUMBER

	long rachilla hairs	short rachilla hairs	t	df	P
F ₂ pooled	6.357	5.214	2.951	683	***
M ₂ GPxSl38					
-500rads	6.379	5.191	3.467	632	***
-1000rads	7.303	6.500	1.184	348	
M ₂ Sl38xGP					
-500rads	6.669	5.072	3.491	502	***
-1000rads	6.811	5.171	1.881	194	
	rough awns	smooth awns	t	df	P
F ₂ pooled	6.172	5.627	1.471	681	
M ₂ GPxSl38					
-500rads	6.240	5.685	1.709	629	
-1000rads	7.274	6.367	1.343	350	
M ₂ Sl38xGP					
-500rads	6.310	6.176	0.297	502	
-1000rads	6.906	5.638	1.600	191	
	tall	dwarf	t	df	P
F ₂ pooled	6.218	5.661	1.330	686	
GPxSl38					
-500rads	6.176	6.121	0.155	625	
-1000rads	7.377	6.426	8.568	345	***
M ₂ Sl38xGP					
-500rads	6.313	5.962	0.754	502	
-1000rads	6.841	5.569	1.736	194	

Table 15: Summary of tiller number/major gene linkage by treatment

The families shown in Table 16 are those in which significant differences between phenotypes were recorded. In no case were these differences significant for both reps, perhaps because of the small sample size. But neither were there any families with a positive significant difference in one rep yet a negative significant difference in the other.

No particular pattern of linkage with dose or direction of cross emerged. Of the 14 cases set out in Table 16, 11 displayed opposite linkage to that generally exhibited i.e. recessive phenotypes had more tillers than the dominant ones. In one family, GPXS138-500 (7), more tillers were associated not only with smooth awned plants but also with those possessing short rachilla hairs. Both these characters are recessive and inherited from S138 which, in this instance, was the irradiated pollen donor.

In contrast to their increased scores for tiller number, dominant phenotypes tended to be shorter than their recessive relations (Table 17). In the case of rachilla hair type, the difference in height was significant in the F_2 and in 3 of the 4 irradiated treatments. And, while it wasn't significant for awn type, the tendency was evident in all but one of the treatments.

The exception to the rule was S138XGP at 1000 rads. Here dominant, rough-awned plants were taller than their smooth-awned counterparts. Interestingly, the dominant Golden Promise, which in this instance was the irradiated parent, was also the shorter parent.

When M_2 families were examined individually, only 7 exhibited significant differences in height between phenotypes (Table 18). All followed the general tendency for taller recessive phenotypes.

Ear length, like tiller number, was greater in the dominant phenotypes. For both rachilla hair type and awn type, the difference between phenotypes was significant in the F_2 . While in the M_2 , linkage had broken down in some cases yet remained significant in others (Table 19).

	R-r		S-s		Ert-ert	
	+	-	+	-	+	-
GXS-500	-	7,15	16,24	7	-	-
-1000	-	-	-	4,7	-	-
SXG-500	-	2,6	-	13	-	4,12
-1000	-	-	7	-	-	-

Table 16: M_2 families in which there were significant differences in tiller number between dominant and recessive phenotypes

HEIGHT (cm)

	long rachilla hairs	short rachilla hairs	t	df	P
F ₂ pooled	67.909	70.217	-2.724	683	**
M ₂ GPxS138					
-500rads	67.960	71.093	-3.540	632	***
-1000rads	66.254	69.657	-2.698	348	**
M ₂ S138xGP					
-500rads	67.925	71.392	-3.647	501	***
-1000rads	67.557	70.000	-1.327	194	
	rough awns	smooth awns	t	df	P
F ₂ pooled	68.168	69.176	-1.156	681	
M ₂ GPxS138					
-500rads	68.244	69.854	-1.923	629	
-1000rads	66.627	68.797	-1.703	350	
M ₂ S138xGP					
-500rads	68.633	68.964	-0.323	502	
-1000rads	68.753	65.277	2.126	191	*

Table 17: Summary of height/major gene linkage by treatment

	R-r		S-s	
	+	-	+	-
GXS-500	-	13	-	15
-1000	-	-	-	7
SXG-500	-	4	-	5,13,14
-1000	-	-	-	-

Table 18: M_2 families in which there were significant differences in height between dominant and recessive phenotypes

EAR LENGTH (cm)

	long rachilla hairs	short rachilla hairs	t	df	P
F ₂ pooled	7.533	7.088	4.249	683	***
M ₂ GPxS138					
-500rads	7.537	7.434	0.934	632	
-1000rads	7.441	7.433	0.049	348	
M ₂ S138xGP					
-500rads	7.487	7.164	2.688	494	**
-1000rads	7.492	7.485	0.035	219	
	rough awns	smooth awns	t	df	P
F ₂ pooled	7.510	7.191	3.017	681	***
M ₂ GPxS138					
-500rads	7.541	7.539	0.020	629	
-1000rads	7.575	7.398	1.055	350	
M ₂ S138xGP					
-500rads	7.344	7.151	1.575	502	
-1000rads	7.574	7.192	2.141	191	*

Table 19: Summary of ear length/major gene linkage by treatment

When individual M_2 families were examined (Table 20), 11 displayed significant differences between phenotypes. Again no dose or cross related pattern emerged; in 8 families dominant types had longer ears than their recessive relatives, while in 3 the reverse situation applied.

	R-r		S-s	
	+	-	+	-
GXS-500	11,24	-	9	-
-1000	-	7	-	-
SXG-500	14,17	6	8,11	5
-1000	5	-	-	-

Table 20: M_2 families in which there were significant differences in ear length between dominant and recessive phenotypes

Discussion

The first aim of this study was to see if the maternal trends reported following crosses with irradiated pollen could be repeated. And the second was to establish the relative importance of mutational damage and genomic selection in determining the M_2 phenotype. Thereby it was hoped a useful assessment of the value of pollen irradiation to plant breeders could be made. Unfortunately, the results of this study were far from conclusive.

- the first generation

Despite parental differences, the M_1 was relatively uniform and similar to the F_1 in appearance. Seed set was the only continuously varying character to be affected by dose. While this characteristic appears to be a feature of pollen irradiation experiments, the degree of similarity between the M_1 and F_1 has varied with species and experiment. In the studies of tomato and peas, for example, irradiated and control progenies were similar in the first generation. Whereas in the earlier barley work and in Nicotiana, the M_1 was more variable and/or maternal than the F_1 .

From a practical point of view, variability in the M_1 would be desirable if it enabled selection to take place at an earlier stage. In past experiments where the M_1 has been variable, however, M_1 phenotype hasn't been a good indicator of the M_2 phenotype. So it's unlikely that effective selection could take place before the second generation.

Since the M_1 in this study was like the F_1 , a substantial amount of paternal information had been transferred from the irradiated pollen. So neither of Pandey's proposed mechanisms can have been in operation. Nor were there any phenotypically aberrant plants showing the typically deleterious effects of chromosome damage like those described by Werner and Cornish in Nicotiana.

- cytology

34% of the M_1 plants examined displayed structural rearrangements, but none were aneuploid. As some plants produced quadrivalents revealing reciprocal translocations that hadn't been apparent at mitosis, and because some plants were only screened at mitosis, this is probably an underestimate. But it is close to the 31% obtained in barley by Borrino *et al.*, and it is in keeping with the relatively normal appearance of the M_1 hybrids.

Although the incidence of visible chromosome damage is low when compared to wheat where Snape *et al.* found most plants exhibited structural damage and aneuploidy, and to *Nicotiana rustica* where the incidence was 86% [Werner *et al.*, 1984], barley is the only diploid species of the three. As such it may be less tolerant of structural/numerical change. In which case, less damage would be expected to persist to the M_1 generation anyway.

- quantitative characters

In order to establish the relative importance of mutational damage and genomic selection, reciprocal irradiated crosses were performed. When Cornish and Werner [1985] carried out similar crosses in *Nicotiana*, they found a consistent trend for reduced vigour irrespective of the direction of the cross. By contrast, in this experiment with barley, wherever a reduction in vigour occurred it was consistent with a maternal trend.

This was the situation for height, where both parents were shorter than the F_2 and trends were in a downward direction. Arguably, these trends may reflect a loss of gene function as a result of radiation damage. Were this to have been the case, however, an excess of low scoring phenotypes would have been expected. In fact, the shape of the distribution of height in the M_2 was very similar to that in the F_2 .

As a measure of fertility, tiller number would be expected to be particularly susceptible to radiation damage. The F_2 produced more tillers than one parent and an equivalent number to the other. Surprisingly then, where there was a shift it was for increased tiller number - a result which runs counter to that expected for either of the proposed mechanisms.

Ear length was the only character in which the F_2 was intermediate, and so the only one where opposite trends could be demonstrated. One group of M_2 s did have longer ears and the other group shorter ears than the F_2 , but neither shift appeared dose-dependent.

The last quantitative character to be considered was awn length, the average for the F_2 being equal to that of one parent and greater than that of the other. If genomic selection were key, a trend would only be expected in one direction of the cross and it would be for decreasing awn length. In this group there was indeed a trend, but it was opposite to that expected.

To summarise, even though maternal trends were not always apparent, there certainly wasn't a consistent trend for reduced vigour either. The M_2 and F_2 distributions of quantitative characters (including those associated with fertility) were similarly shaped, with no excess of low scoring phenotypes in the irradiated progenies. So it seems that in this experiment with barley, the persistence of radiation induced damage was not a major determinant of M_2 phenotype.

Owing to the non-targeted nature of the radiation treatment, M_1 plants might be affected in different ways. This could become apparent in the M_2 generation in the form of significant differences between families. And, indeed, such differences did occur, although not in every treatment for every character. Where differences were not significant, it was probably due to the relatively small numbers of degrees of freedom, rather than an indication that parts of the genome had been preferentially affected (there having been no suggestion of this in earlier studies).

By contrast, no significant differences between the F_2 families would have been expected. Reality proved somewhat different to expectation for the GPXS138 F_2 . Because, in this case, awn length was the only character in which there weren't significant differences between families. A comparison of the analysis of variance results for the reciprocal F_2 s [Appendix A1] shows that while the error mean squares for both controls were of a similar magnitude, the between mean squares were notably larger in the F_2 GPXS138. The family scores for this group are presented below.

	ht	tn	el	mean rank
1.	70.6	5.4	7.2	5.0
2.	76.4	7.9	8.3	1.0
3.	67.4	5.9	7.0	6.7
4.	65.6	4.2	7.1	8.3
5.	66.0	6.5	7.4	5.0
6.	72.2	4.6	7.2	5.3
7.	62.5	6.6	8.0	5.0
8.	68.4	6.9	7.1	4.7
9.	69.2	5.8	7.5	4.3
10.	64.3	4.9	6.3	9.0

Family 2 was consistently high scoring, while families 4 and 10 were generally low scoring. These differences did not appear to be parental in origin, since the 3 F_1 plants from which these families were derived achieved similar scores (in fact, the plant which was marginally highest scoring gave rise to the lowest scoring family). Neither were there any differences in the way first generation plants were treated.

It is possible that plants or seeds were mislabelled somewhere along the line, so confusing irradiated and control material. But as M_1 seed was generally shrivelled while F_1 seed was plump, and as there was usually much more F_2 than M_2 seed, this explanation is perhaps unlikely. Since reps were prepared for planting in turn, and the anomalous families were consistent over reps, it doesn't seem likely that seed was taken from the wrong packets either. As the parents also appeared to have bred true, these results remain a mystery.

- qualitative characters

As far as major genes are concerned, all earlier irradiated pollen experiments have resulted in an excess of maternal types in the M_2 generation (albeit of varying size). According to the proponents of genomic selection, this is largely the result of selection against radiation induced damage during the production of the M_2 . The maternal genome is therefore represented to a greater extent than the paternal genome in this generation. Supporters of the mutational damage theory, on the other hand, would argue that most paternal alleles are present in the M_2 but that their expression is impaired or they are totally inactivated. So some non-parental types might also be expected in the M_2 . In either event, there should be a relative increase in the number of maternal types compared to the number of paternal types. In view of this, the results obtained in this experiment are surprising.

The pooled segregation ratios are set out below. There were no non-parental types.

	R:r	S:s	Ert:ert
GP	1:0	1:0	0:1
S138	0:1	0:1	1:0
F_2	2.87:1	3.01:1	3.30:1
M_2 GXS	3.46:1	3.92:1	2.86:1
M_2 SXG	4.11:1	3.83:1	3.17:1

When linkage between quantitative and qualitative characters was examined, M_2 families were found in which plants exhibiting paternal major gene traits achieved higher scores than their maternal-type counterparts. Again if much mutational damage had persisted, one might expect reduced scores in those plants inheriting paternal characters, especially where these were of a recessive nature. When compared to the overall linkage patterns in the F_2 , M_2 examples of linkage breaking down, forming and also reversing were found.

- conclusion

When this study was set up, it had already been established that gametophytic selection would have to play a greater part in determining M_2 phenotype than the persistence of radiation induced damage if pollen irradiation was to have a useful application in plant breeding. It is clear that in this experiment considerable deleterious radiation damage did not persist, confirming earlier reports of the effects of pollen irradiation in barley. But unfortunately, the previously observed maternal trends were not as evident in this study either.

Perhaps the lack of consistent trends is in part a reflection of the lower doses used, Powell *et al.* [1983] having recovered plants at doses of up to 2000 rads. However, to achieve these results embryo rescue techniques were required which add to the complexity of the procedure. What's more the extra effort involved in producing plants at higher doses didn't seem to have paid great dividends, at least in this study.

A comparison of M_2 and F_2 results is presented in Table 21 from which it can be seen that significant departures from the F_2 occur at roughly the same frequency in both the 500 and 1000 rad treatment groups. Interestingly, a visibly abnormal M_1 mitosis or meiosis didn't lead to reduced vigour in the second generation suggesting some elimination of the damaged paternal genome had occurred. What's more, a disturbed linkage pattern or segregation ratio wasn't associated with lower quantitative scores either.

Unexpectedly, deviations from the F_2 in both crosses were in the same direction. For two genes there was an excess of dominant types, and for the third an excess of recessive types. In all cases this meant the excess was of GP-types (so the results for the GPXS138 cross are as expected). Had there been significant differences between the reciprocal F_2 s it may have helped to explain these findings. But as there weren't, they remain mysterious especially in light of the quantitative results (where the M_2 S138XGP irradiated cross produced results consistent with increasing maternalisation just as often as its reciprocal M_2 did).

At the family level, the results were equally confusing with almost as many significant departures in favour of paternal types occurring as those favouring maternal types (6 vs 9 cases). While it's true to say the degree of maternalisation of the M_2 has varied in previous studies, none of these have reported such widespread paternalisation.

- linkage

When the M_2 segregation ratios of linked genes were considered, much of the departure from the F_2 was due to disturbances in the ratios of individual genes involved. But there were also cases where individual ratios were consistent with those of the F_2 , and the departure seemed to be the result of increased recombination. Such an increase may be of benefit to the plant breeder in cases where the desired phenotype is a rare recombinant. And it may also help to break down those linkages which survive conventional crossing.

As far as continuously varying characters were concerned, parental correlation coefficients were generally positive and low with the F_2 distribution centering on these values. If mutational damage had been an important determinant of the M_2 phenotype, the value of 'r' would have been expected to remain positive but to increase in magnitude. That is, there would have been an association between a low score for one character and a low score for another because of widespread mutational damage. In fact, the striking feature of the M_2 was the downward extension of correlation coefficients. So once again, mutational damage doesn't appear to have been key.

family	cytology ^a	qualitative traits ^b	quantitative traits ^c	linkage ^d
1		*	---+	-*-
2		-	+==+	*--
3		-	---+	-*-
4		*	---+	-*-
5	*	-	---=	----
6		-	+++	----
7	*	-	-++	---*
8		-	-+-	----
9		-	+--	---*
10		-	---+	----
11		-	-+-	---*
12		-	+--	-*-
13		-	+--	---*
14		-	+++	----
15		-	+++	---
16		*	+++	*--*
17	*	-	+++	-*-
18		-	-+-	-*-
19		-	+++	-*-
20		*	+++	----
21	*	-	-+=	*--
22		-	---	----
23		-	+++	----
24		-	-++	---*
25		-	---+	----

KEY

a * = abnormal mitosis and/or meiosis

b * = significant departure from the F₂ for a major gene segregation ratio

c = comparison with F₂-height, tiller number & ear length

d * = pattern of linkage significantly different to that of the F₂-qualitative characters, quantitative characters and qualitative/quantitative characters

Table 21: Comparison of M₂ with F₂ results

M₂ GPXS138-1000rads

family	cytology ^a	qualitative traits ^b	quantitative traits ^c	linkage ^d
1	*	-	-++	**_
2		*	-++	*_
3	*	-	-+-	_*
4		*	+++	*_*
5		-	-++	---
6		*	+++	**_
7		-	-++	_*
8	*	*	-++	---
9		-	-+-	---
10		-	+++	---
11		-	---	_*
12	*	*	-++	**_

Table 21 continued

M₂ S138XGP-500rads

family	qualitative traits ^b	quantitative traits ^c	linkage
1	-	+--+	-*-
2	-	-+-	__**
3	-	+++	---
4	-	+--+	__**
5	-	-+-	__**
6	-	-+-	__*
7	*	++-	-*-
8	*	+--+	***
9	-	++-	-*-
10	*	-+-	---
11	-	---	__*
12	-	-++	__**
13	-	-+-	__*
14	-	---	__**
15	-	-+-	---
16	-	+--	---
17	-	---	-*-
18	-	+--+	-*-

Table 21 continued

M₂ S138XGP-1000rads

family	qualitative traits ^b	quantitative traits ^c	linkage ^d
1	-	-+-	----
2	-	=-+	----
3	-	-++	-*-
4	-	---	*--
5	-	+++	-**
6	-	-+-	----
7	-	++-	*--*

Table 21 continued

Because of the lack of consistent maternal trends in this study, yet their occurrence in earlier barley work, it is difficult to predict the potential advantage of pollen irradiation over backcrossing. There is possibly some benefit in that linked genes may be separated more easily. And the frequency of rarer recombinants may also be increased. Moreover, the generally greater variability released by the radiation treatment may broaden the range of plants from which selection can take place.

It is conceivable that a programme of low dose irradiated pollen crossing could be undertaken in barley as a supplement to conventional backcrossing without requiring a great deal of extra input. Were the technique to achieve the desired results, the actual mechanism involved would assume less importance!

PART 3

IRRADIATED OVULE CROSSES IN BARLEY

Introduction

Experimental Design

Method

- crossing procedure
- the glasshouse stage
- the field stage
- scoring the second generation

Results

- the first generation
- the second generation : quantitative characters
- the second generation : qualitative characters
- linkage study : quantitative characters
- linkage study : qualitative characters

Discussion

- the first generation
- quantitative characters
- qualitative characters
- linkage
- conclusion

Introduction

While the efficacy of pollen irradiation as a technique in plant breeding had been evaluated in a number of species, there had been no reported studies in which the female rather than the male gamete had been irradiated. So whether paternal trends could be induced by irradiating ovules (as maternal trends had been by irradiating pollen) was a question still to be answered.

As a technique, ovule irradiation may offer some advantages over the pollen alternative. Once a pollen grain has germinated, the pollen tube must grow down the style to effect fertilisation. The ovule, on the other hand, simply awaits the arrival of sperm cells. So it's likely that the male gamete will be more sensitive to radiation damage than its female counterpart. This may enable viable M_1 seed to be collected at higher doses when the female gamete is irradiated, hopefully further limiting the contribution that this parent makes to subsequent generations.

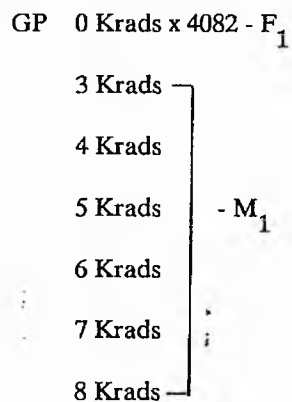
In order to assess the potential of ovule irradiation as a means of limited gene transfer, an experiment with barley was set up in 1984.

Experimental design

Irradiated and control crosses were made between two highly inbred lines of spring barley: the cultivar Golden Promise, and the marker stock 4082. These lines differ in a number of quantitative and qualitative characters; the major gene differences utilised in this experiment are given below.

GP	4082	chromosome number
N - non naked caryopsis	n - naked caryopsis	1
V - 2 row	v - 6 row	2
O - white lemma	o - orange lemma	6
R - rough awn	r - smooth awn	7
S - long rachilla hairs	s - short rachilla hairs	7

Crosses were made according to the following schedule:-



The F_1 and M_1 were raised in the glasshouse and selfed to produce the F_2 and M_2 which were field grown. Each generation was scored for both quantitative and qualitative characters.

Method

(Apart from the following differences, the methods used are the same as those detailed in the irradiated pollen section.)

- crossing procedure

Some 2 to 3 days after emasculation, GP plants were transported to the Western General Hospital, Edinburgh, where emasculated ears were given x-ray doses of between 0 and 8 Krads. They were immediately pollinated with fresh 4082 pollen. Plants were then returned to the glasshouse where they remained until ready to be harvested.

- the glasshouse stage

The resulting seeds were germinated in Petri dishes and the seedlings potted on. Plants were raised in a randomised block design in the glasshouse during the summer of 1984.

At maturity, plants were scored for major gene characters in addition to height, ear length, the number of seed on the main tiller and the bulk seed number.

- the field stage

The F_2 , M_2 and both parents were field-grown in a replicated randomised complete block design at Pentlandfield, Midlothian, in 1985. The number of families raised for each treatment is given below:

F_2 0 Krads - 10

M_2 3 Krads - 17

M_2 4 Krads - 10

M_2 5 Krads - 3

M_2 6 Krads - 8

M_2 7 Krads - 5

M_2 8 Krads - 1

Each family was represented in each of three blocks by a row of up to 20 plants.

- scoring the second generation

The continuously varying characters scored were:-

- height
- fertile tiller number
- green tiller number
- ear length
- awn length

These characters were measured in 5 randomly selected plants from each row.

The major genes, which were scored for each plant, were:

- non-naked (N) vs naked (n) caryopsis
(in individuals homozygous for n, the palea and lemma were easily removed from the seed)
- 2-row (V) vs 6-row (v)
- white (O) vs orange (o) lemma
- rough (R) vs smooth (r) awns
- long (S) vs short (s) rachilla hairs

Results

(Where possible data have been analysed as they were in the irradiated pollen experiment. For full details see Part 2).

- the first generation

Both irradiated and control hybrids had non-naked caryopses, 2 rows, white lemmas, rough awns and long rachilla hairs which confirmed the dominance relationships. The M_1 tended to be taller than the F_1 , although a trend with dose was not apparent (Table 22). Ear length was similar for all groups apart from the 5 Krad M_1 in which ears were appreciably shorter. As in the irradiated pollen studies, seed set fell with increasing radiation dose. While the M_1 was generally more variable than the F_1 , there were no grossly abnormal irradiated hybrid plants.

- the second generation : quantitative characters

i height

The analysis of variance in height is summarised in Table 23 (see also Appendix B1). By testing each within families mean square against the environmental mean square, a significant genetic component in the variation for height was identified. The only treatment in which there were significant differences between families was the 3 Krad group.

The differences between treatments are displayed in Figure 28 (histograms of second generation family height in which each datum is the mean of up to 60 plants), in Figure 29 (where family variances are plotted according to treatment), and in the following table (where means and standard errors of the means for height are given).

Table 22: Summary of quantitative data from the F₁ and M₁ generation

genotype	number of plants	height <u>+</u> SEM	ear length <u>+</u> SEM	seed set <u>+</u> SEM
GP X 4082	35	78.26 <u>+</u> 1.46	8.06 <u>+</u> 0.18	143.48 <u>+</u> 7.23
-3KradS	17	101.4 <u>+</u> 1.70	9.12 <u>+</u> 0.35	128.24 <u>+</u> 14.96
-4KradS	10	90.50 <u>+</u> 4.27	9.30 <u>+</u> 0.40	81.30 <u>+</u> 12.83
-5KradS	3	85.33 <u>+</u> 3.18	5.67 <u>+</u> 0.33	52.33 <u>+</u> 9.59
-6KradS	8	98.63 <u>+</u> 3.03	8.63 <u>+</u> 0.56	99.00 <u>+</u> 16.77
-7KradS	5	89.80 <u>+</u> 8.83	8.00 <u>+</u> 1.18	35.20 <u>+</u> 7.79
-8KradS	1	93.00	8.00	53.00

Table 23 : Analysis of variance - height

	\bar{x}	within families			between families		
		MS	df	P	MS	df	P
GP	60.05	11.694	36	-	66.094	2	
4082	86.13	0.658	36	-	125.365	2	
<hr/>							
F2	79.02	94.412	120	**	160.706	9	
M2-3KradS	82.96	130.074	196	**	431.118	15	***
M2-4KradS	80.25	164.076	114	**	197.766	4	
M2-5KradS	79.83	128.847	32	**	66.667	2	
M2-6KradS	80.29	100.915	92	**	44.286	7	
M2-7KradS	80.20	103.662	40	**	168.685	4	
M2-8KradS	82.93	66.01	12	**	-	-	-

- = non-applicable
 = non-significant
 * = $P < 0.05$
 ** = $P < 0.01$
 *** = $P < 0.005$

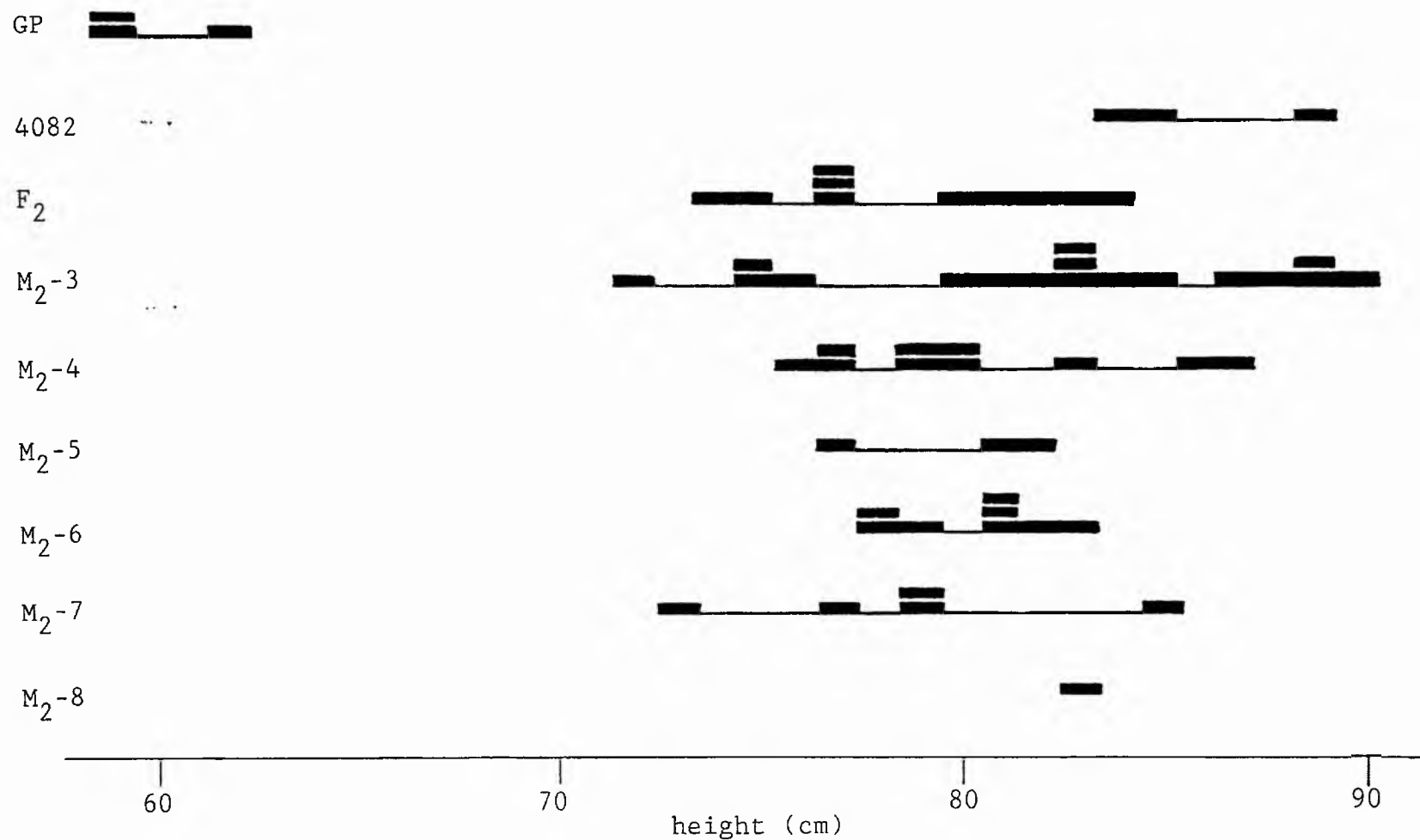
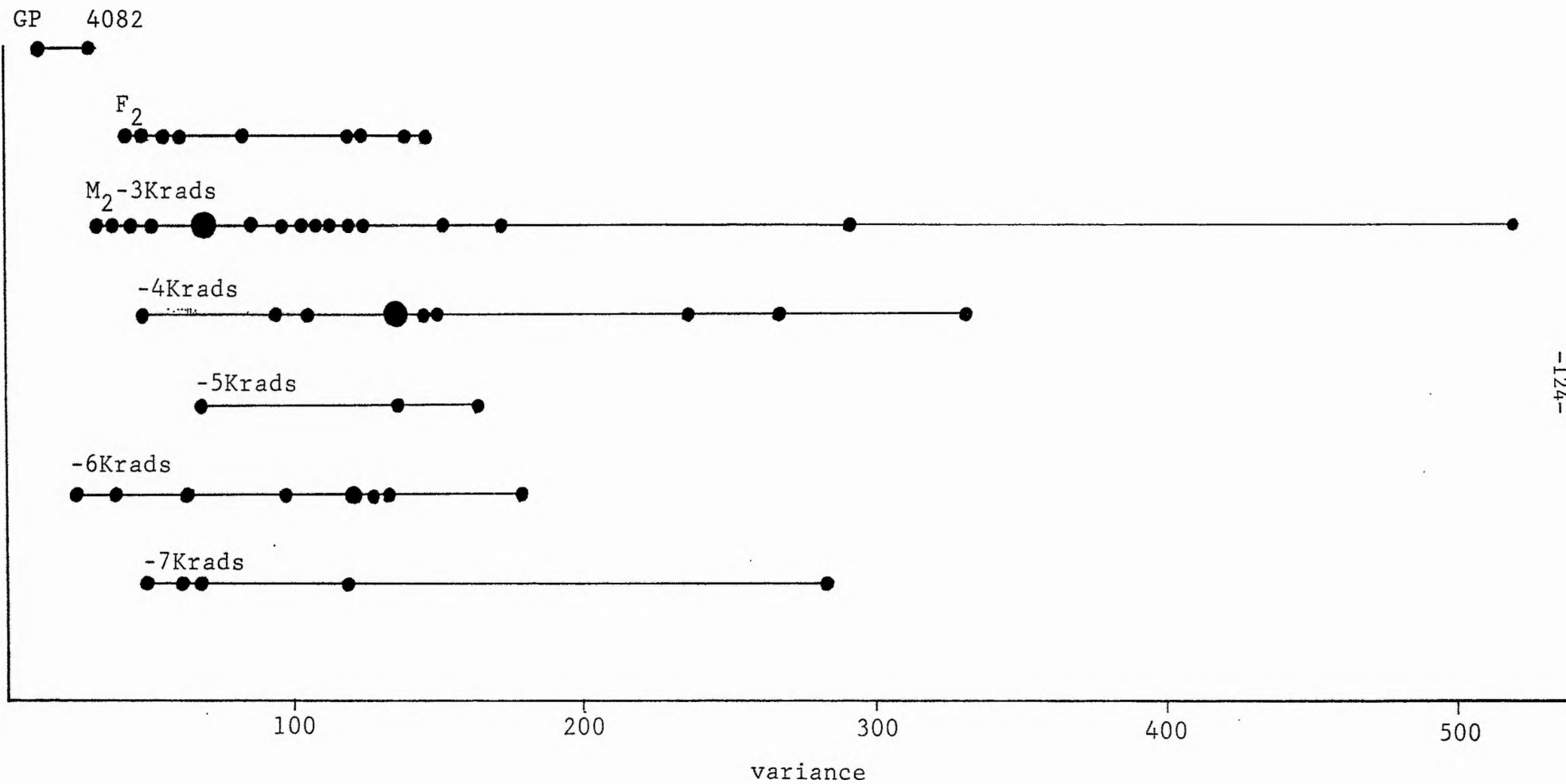


Figure 28 : Distribution of second generation family heights



1 ● 2 ● 3 ● 4 ● 5 ●
number of families

Figure 29 : Height variability within second generation families

GP	60.06 \pm 0.51
4082	86.13 \pm 0.12
F ₂	79.02 \pm 0.79
M ₂ -3	82.96 \pm 0.73
-4	80.25 \pm 1.07
-5	79.83 \pm 1.66
-6	80.29 \pm 0.93
-7	80.20 \pm 1.37
-8	82.93 \pm 2.10

GP was shorter than the F₂ which was shorter than 4082. An increase in M₂ height relative to the F₂ would, therefore, have been consistent with a shift towards the paternal parent. While the M₂ means were indeed larger than that of the F₂, this phenomenon was only statistically distinguishable in one case and a dose-dependent trend was not discernible.

When it came to the distribution of family means, the spread of the M₂ was greater than that of the F₂ (Figure 28). But while only two M₂ families were lower scoring than the lowest F₂, eight had higher means than the highest F₂ (so putting them in the range of the paternal parent). The variability within most M₂s was similar to that exhibited by the F₂s, although there were some exceptionally variable M₂ families (Figure 29).

ii. tiller number

The analysis of variance in tiller number is summarised in Table 24 (see also Appendix B1). There were significant genetic differences within families for all treatments, but no significant differences between families within treatments.

Table 24 : Analysis of variance - tiller number

	\bar{x}	within families			between families		
		MS	df	P	MS	df	P
GP	5.31	6.411	36	-	4.823	2	
4082	3.11	4.722	36	-	0.289	2	
<hr/>							
F ₂	5.65	16.457	120	**	20.635	9	
M ₂ -3KradS	5.70	29.098	196	**	36.874	15	
M ₂ -4KradS	5.59	13.603	114	**	15.735	4	
M ₂ -5KradS	5.02	8.925	32	*	15.560	2	
M ₂ -6KradS	5.67	10.191	92	**	11.724	7	
M ₂ -7KradS	7.16	14.045	40	**	48.665	4	
M ₂ -8KradS	6.67	43.600	12	**	-	-	-

- = non-applicable
 = non-significant
 * = P < 0.05
 ** = P < 0.01
 *** = P < 0.005

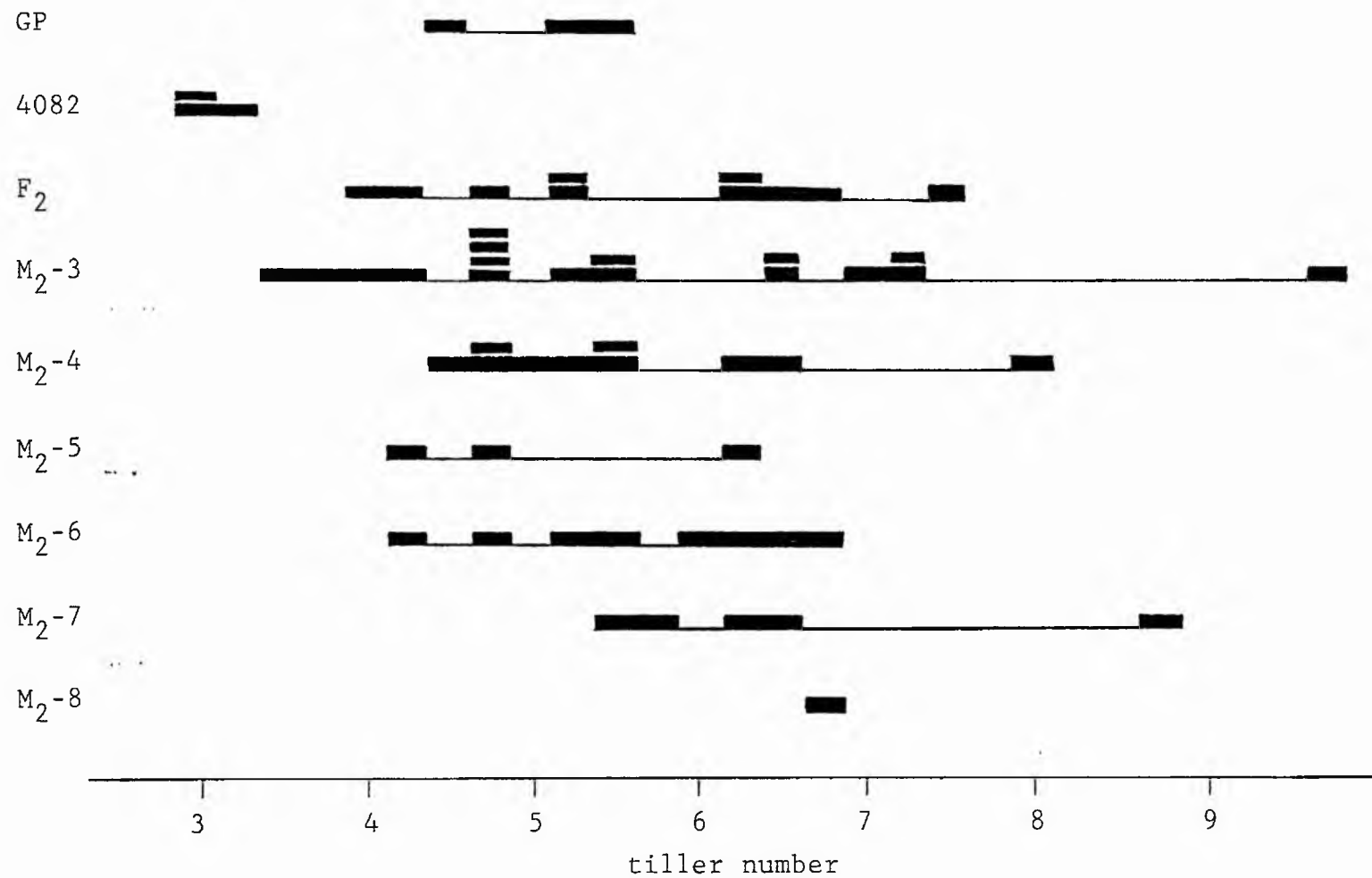
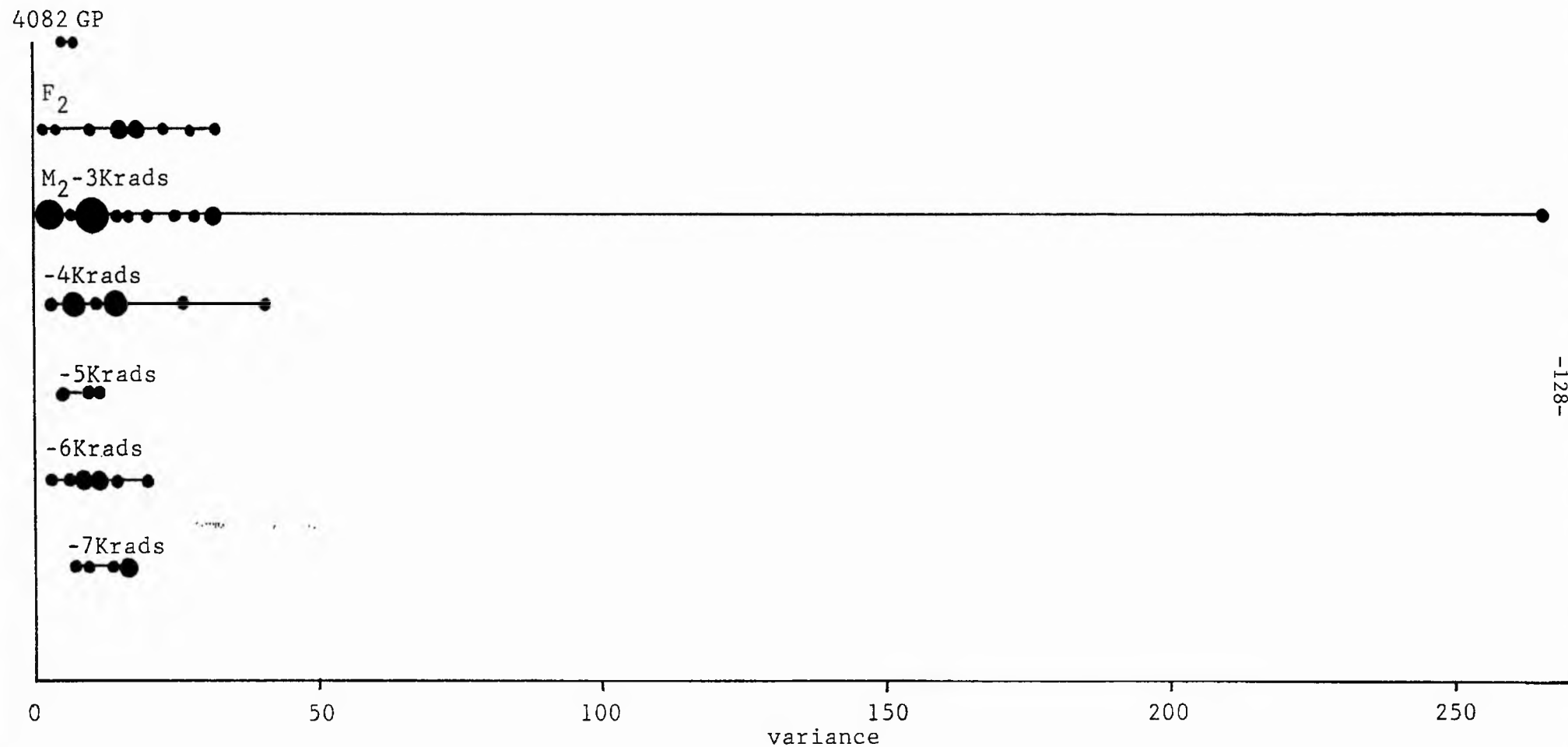


Figure 30 : Distribution of second generation family tiller numbers



1 ● 2 ● 3 ● 4 ● 5 ●
number of families

Figure 31 : Tiller number variability within second generation families

The means and standard errors of the means are presented below:-

GP	5.31 \pm 0.38
4082	3.11 \pm 0.32
F ₂	5.65 \pm 0.33
M ₂ -3	5.70 \pm 0.34
-4	5.59 \pm 0.31
-5	5.02 \pm 0.44
-6	5.67 \pm 0.30
-7	7.16 \pm 0.51
-8	6.67 \pm 1.70

4082 produced fewer tillers than GP which was statistically indistinguishable from the F₂. Intriguingly, where there was a significant departure from the F₂ it was for increased tiller number.

The M₂ and F₂ histograms (Figure 30) were similar with only five M₂ families falling outside the F₂ limits (two had a lower, and three a higher, mean tiller number). This similarity was echoed in the variance distribution where only one odd M₂ family was evident (Figure 31). With a variance more than five times as large as any other family, however, this 3 Krad family was highly exceptional.

iii. ear length

The analysis of variance in ear length (Table 25, see also Appendix B1) reveals significant genetic variation within families for all treatments. There were significant differences between families within the 3 Krad and 5 Krad treatments.

Table 25 : Analysis of variance - ear length

	\bar{x}	within families			between families		
		MS	df	P	MS	df	P
GP	8.03	0.658	36	-	0.199	2	
4082	6.77	0.669	36	-	0.118	2	
<hr/>							
F ₂	7.80	1.616	120	**	2.428	9	
M ₂ -3KradS	7.78	1.821	196	**	3.643	15	*
M ₂ -4KradS	7.58	2.660	114	**	4.116	4	
M ₂ -5KradS	7.70	1.019	32	*	5.068	2	*
M ₂ -6KradS	7.60	1.247	92	**	2.218	7	
M ₂ -7KradS	7.95	1.424	40	**	3.695	4	
M ₂ -8KradS	8.03	2.600	12	**	-	-	-

- = non-applicable
 = non-significant
 * = p < 0.05
 ** = p < 0.01
 *** = p < 0.005

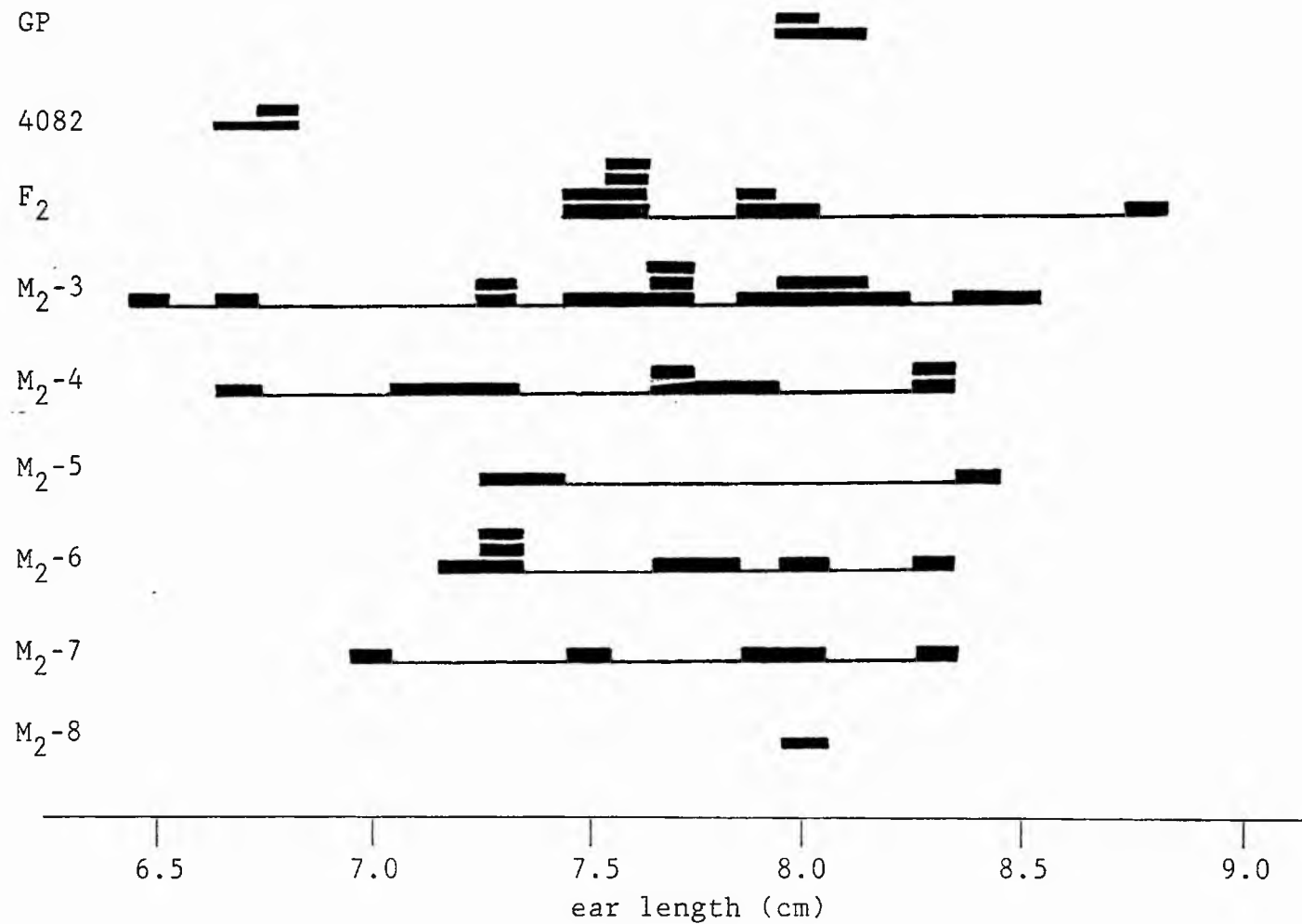
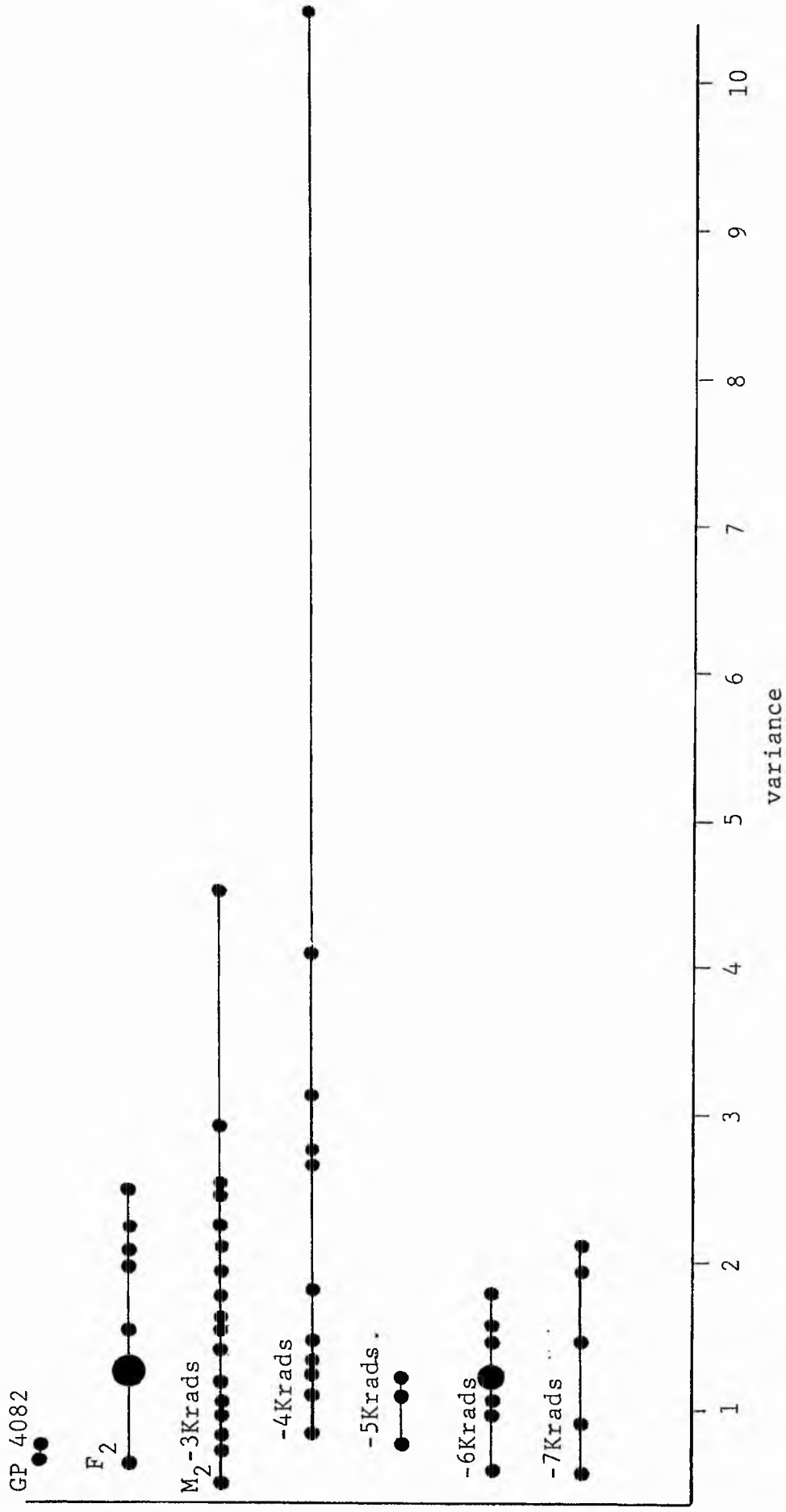


Figure 32 : Distribution of second generation family ear lengths



1 ● 2 ● 3 ● 4 ● 5
number of families

Figure 33 : Ear length variability within second generation families

While the means and standard errors of the means show up significant parental differences, the F_2 and the M_2 once again resembled each other:

GP	8.03 ± 0.12
4082	6.77 ± 0.12
F_2	7.80 ± 0.10
M_2 -3	7.78 ± 0.09
-4	7.58 ± 0.14
-5	7.70 ± 0.15
-6	7.60 ± 0.10
-7	7.95 ± 0.16
-8	8.03 ± 0.42

GP had longer ears than 4082, the F_2 being intermediate for this character. None of the M_2 treatment groups were significantly different from the control group.

A slightly different picture emerged when the distribution of family means was examined (Figure 32). Because, while no M_2 had a mean higher than that of the highest F_2 , 15 had means which were lower than the lowest F_2 . This meant that the range of the paternal parent lay within that of the M_2 . Just as in the case of tiller number, there was one exceptionally variable M_2 family but this time it was derived from the 4 Krad treatment (Figure 33).

iv. awn length

A summary of the analysis of variance in awn length is presented in Table 26 (see also Appendix B1). While there was significant genetic variation within families, significant differences between families within treatments were only observed in the 3 Krad and 7 Krad groups.

Table 26 : Analysis of variance - awn length

	\bar{x}	within families			between families		
		MS	df	P	MS	df	P
GP	12.76	1.114	36	-	0.199	2	
4082	17.80	1.983	36	-	0.948	2	
<hr/>							
F2	17.65	6.802	120	**	6.559	9	
M ₂ -3KradS	18.09	7.261	196	**	13.398	15	*
M ₂ -4KradS	17.69	8.836	114	**	9.254	4	
M ₂ -5KradS	17.20	7.775	32	**	9.877	2	
M ₂ -6KradS	17.81	5.613	92	**	6.483	7	
M ₂ -7KradS	19.19	3.870	40	**	17.095	4	***
M ₂ -8KradS	17.50	8.317	12	**	-	-	-

- = non-applicable
 = non-significant
 * = P < 0.05
 ** = P < 0.01
 *** = P < 0.005

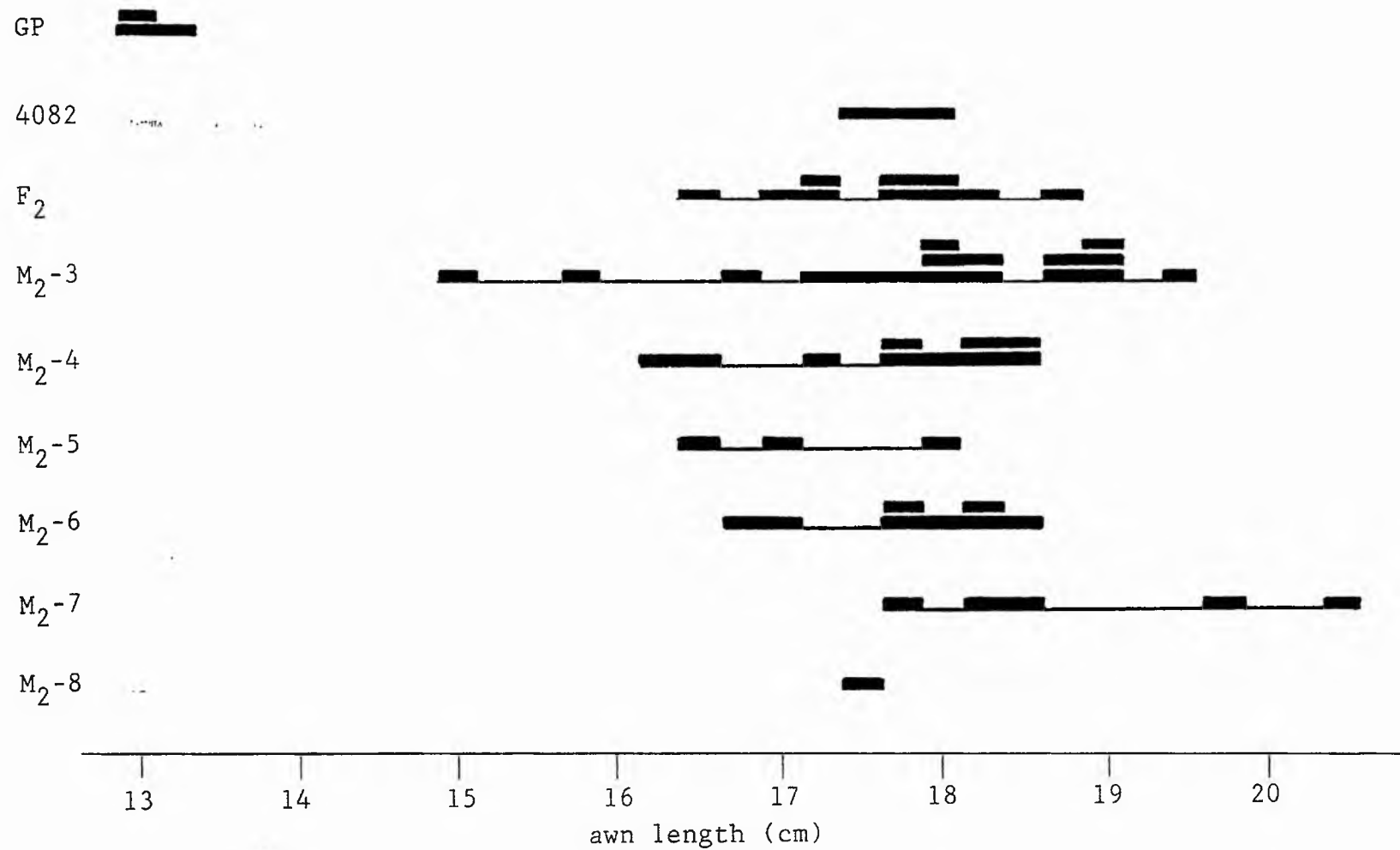


Figure 34 : Distribution of second generation family awn lengths

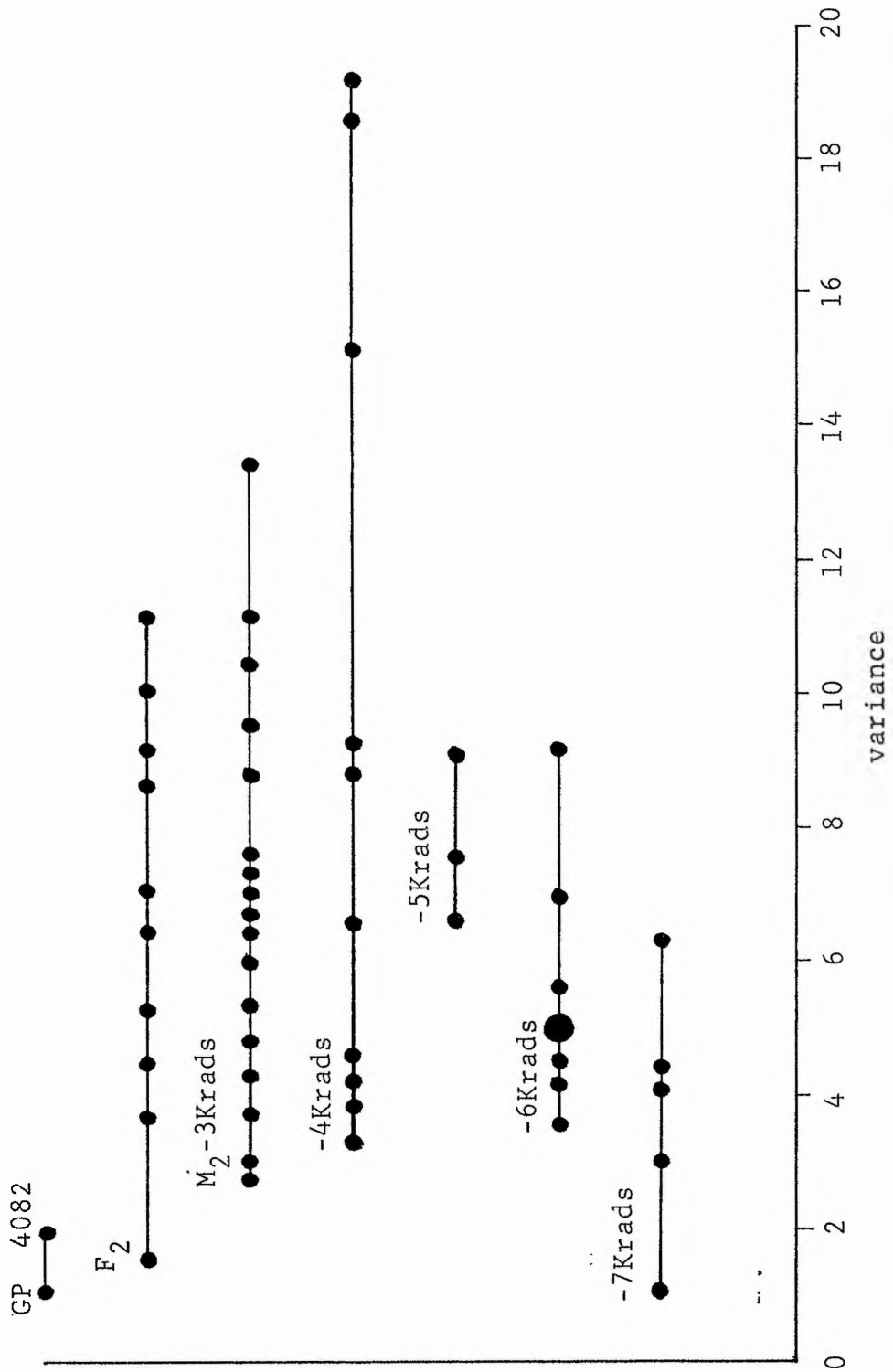


Figure 35 : Awn length variability within 2nd generation families

1 ● 2 ● 3 ● 4 ● 5
number of families

The differences between treatments can be seen in the means and standard errors of the means that follow:

GP	12.76 \pm 0.16
4082	17.80 \pm 0.21
F ₂	17.65 \pm 0.21
M ₂ -3	18.09 \pm 0.25
-4	17.69 \pm 0.41
-5	17.20 \pm 0.22
-6	17.81 \pm 0.22
-7	19.19 \pm 0.27
-8	17.50 \pm 0.74

GP had shorter awns than 4082 which was statistically indistinguishable from the F₂. So, for this character, no paternal trend would have been discernible. Apart from the 7 Krad group, all of the M₂s had a mean awn length equivalent to that of the F₂.

Both second generation distributions centered on that of the paternal parent, 4082 (Figure 34). And, again, most M₂ families fell within the range of variability displayed by their non-irradiated counterparts (Figure 35).

v. green tiller number

The results for green tiller number are presented in Table 27, and in Figures 36 and 37. While there does appear to be a significant genetic component in the phenotypic variation (Table 27), there are several second generation families with a variance lower than that of either parent. In view of the uncertainty of the significance of this character, it was not considered further.

Table 27 : Analysis of variance - green tiller number

	\bar{x}	within families			between families		
		MS	df	P	MS	df	P
GP	2.25	3.311	36	-	3.089	2	
4082	1.00	2.744	36	-	1.800	2	
<hr/>							
F ₂	2.00	4.407	120	**	3.230	9	
M ₂ -3KradS	1.89	3.857	196	**	5.669	15	
M ₂ -4KradS	2.15	4.834	114	**	4.635	4	
M ₂ -5KradS	1.97	4.938	32	**	2.150	2	
M ₂ -6KradS	2.45	4.365	92	**	6.934	7	
M ₂ -7KradS	2.53	5.563	40	*	9.495	4	
M ₂ -8KradS	2.45	7.200	12	**	-	-	-

- = non-applicable
 = non-significant
 * = P < 0.05
 ** = P < 0.01
 *** = P < 0.005

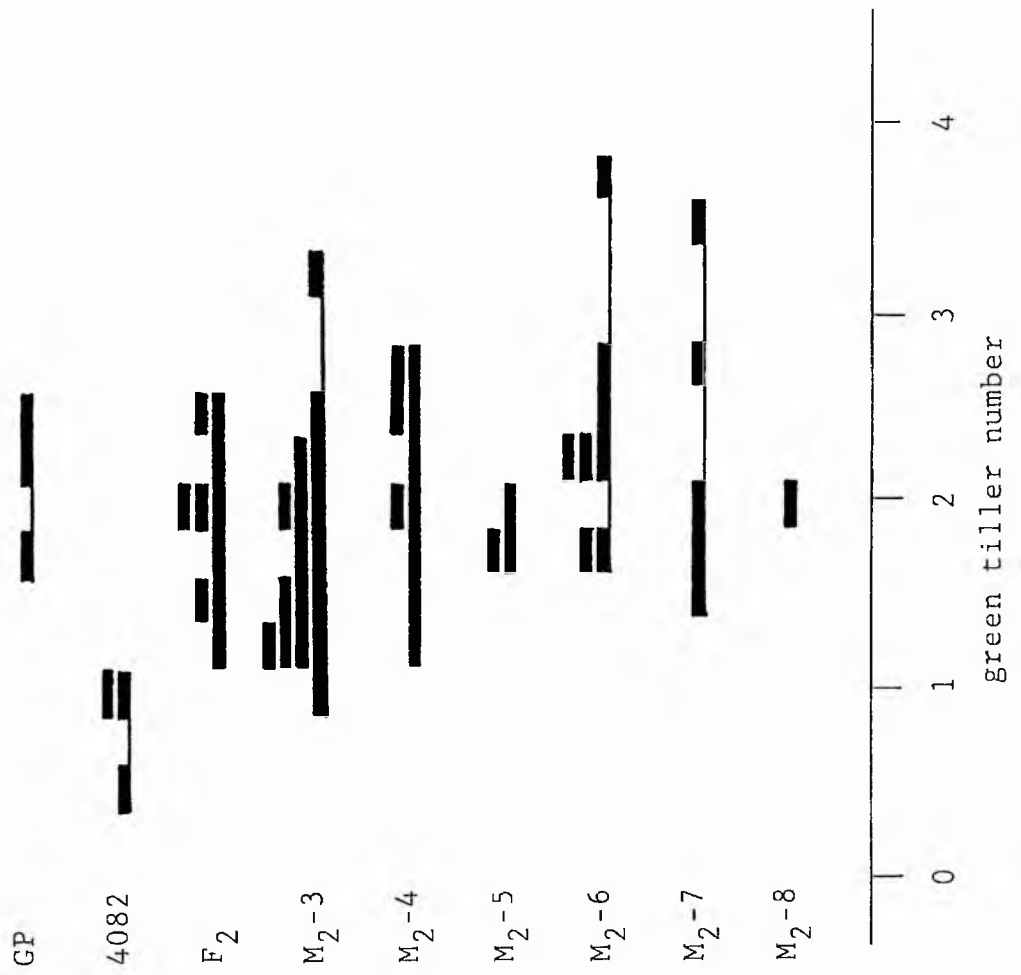
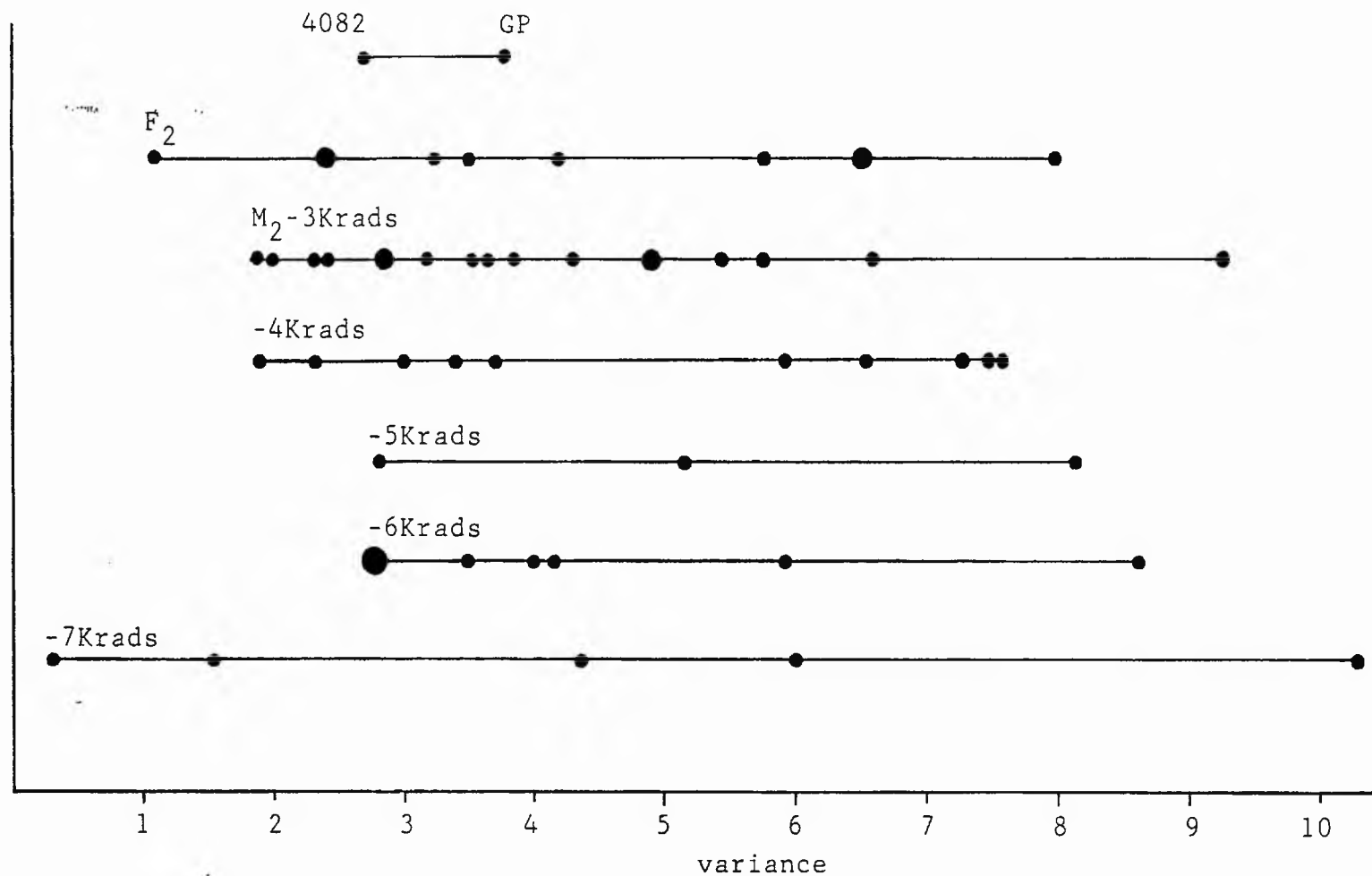


Figure 36 : Distribution of second generation family green tiller numbers



1 ● 2 ● 3 ● 4 ● 5 ●
number of families

Figure 37 : Green tiller number variability within 2nd generation families

- the second generation : qualitative characters

The single gene segregation ratios pooled for each treatment are given in Table 28 together with the results of a heterogeneity χ^2 analysis. While the F_2 was homogeneous for all major gene frequencies, each M_2 displayed significant heterogeneity for at least one of the genes under consideration.

Having examined the homogeneity of treatment groups, the phenotypic frequencies of each M_2 family (and, where families were consistent, of each treatment) were compared with those of the F_2 using the χ^2 goodness of fit test (see Appendices B2 and B3).

i. rough : smooth awn (R : r)

Apart from families derived from the 3 Krad and 6 Krad crosses, the ratio of rough- to smooth-awned plants was consistent for families within treatments. The segregation ratio for each treatment is given below together with the direction of the shift from the F_2 and, where applicable, the significance of the shift.

GP : 4082

F_2	3.38 : 1		
M_2 -3	3.42 : 1	maternal	
M_2 -4	2.88 : 1	paternal	ns
M_2 -5	2.87 : 1	paternal	ns
M_2 -6	3.13 : 1	paternal	
M_2 -7	2.97 : 1	paternal	ns

Apart from the lowest dose treatment, each M_2 produced a higher proportion of recessive paternal types than the F_2 . But where this could be tested, it was not significant and it didn't appear to be dose related either. When M_2 families were considered separately, 5/43 were significantly different to the F_2 for this character. Three of these had an excess of paternal types.

	R:r	P	S:s	P	V:v	P	O:o	P	N:n	P
GP	1:0	-	1:0	-	1:0	-	1:0	-	1:0	-
4082	0:1	-	0:1	-	0:1	-	0:1	-	0:1	-
F_2	3.38:1	0.4083	3.10:1	0.0851	3.24:1	0.9153	2.38:1	0.4030	2.40:1	0.0755
$M_2 - 3$ Krads	3.42:1	0.0391 *	2.79:1	0.6801	2.36:1	0.0000 ***	2.47:1	0.0000 ***	2.60:1	0.0119 *
$M_2 - 4$ Krads	2.88:1	0.1996	2.48:1	0.1154	2.68:1	0.3056	1.91:1	0.0120 *	2.07:1	0.0000 ***
$M_2 - 5$ Krads	2.87:1	0.5169	1.79:1	0.9560	2.16:1	0.0072 **	2.64:1	0.1028	2.13:1	0.1212
$M_2 - 6$ Krads	3.13:1	0.0000 ***	3.23:1	0.1561	1.75:1	0.0007 ***	2.35:1	0.3204	2.66:1	0.2599
$M_2 - 7$ Krads	2.97:1	0.4525	1.24:1	0.2186	2.24:1	0.3063	1.67:1	0.0000 ***	1.67:1	0.0021 ***
$M_2 - 8$ Krads	[2.27:1]	-	[1.77:1]	-	[3.50:1]	-	[0.40:1]	-	[4.14:1]	-

[] - results from only one family

Table 28 : Major gene frequencies in the F_2 and M_2 with the results of heterogeneity χ^2 tests

ii long : short rachilla hairs (S : s)

In the case of rachilla hair type, the χ^2 test of heterogeneity was insignificant in all groups.

GP : 4082

F_2	3.10 : 1		
M_2 -3	2.79 : 1	paternal	ns
M_2 -4	2.48 : 1	paternal	*
M_2 -5	1.79 : 1	paternal	***
M_2 -6	3.23 : 1	maternal	ns
M_2 -7	1.24 : 1	paternal	***

Generally speaking, there was a relative increase in the number of paternal types; a trend that reached significance in three of the four M_2 s. If the results of the 6 Krad treatment are excluded, this excess increased with dose. Seven M_2 families departed significantly from the F_2 , all having relatively more paternal phenotypes. This phenomenon was most marked in the 7 Krad treatment, where four of the five families were significantly more paternal.

iii 2 : 6 rows (V : v)

Only the segregation ratios of those families derived from the 4 and 7 Krad crosses were consistent within treatments.

GP : 4082

F_2	3.24 : 1		
M_2 -3	2.36 : 1	paternal	
M_2 -4	2.68 : 1	paternal	ns
M_2 -5	2.16 : 1	paternal	
M_2 -6	1.75 : 1	paternal	
M_2 -7	2.24 : 1	paternal	ns

Each M_2 had a higher proportion of paternal phenotypes, but where this excess could be tested it was not significant. Neither was it dose-dependent. All 10 of the M_2 families that departed from the F_2 were significantly more paternal.

iv. white : orange lemma (O : o)

The ratio of white to orange lemmas was consistent for families within two of the five irradiated groups.

GP : 4082

F_2	2.38 : 1		
M_2 -3	2.47 : 1	maternal	
M_2 -4	1.91 : 1	paternal	
M_2 -5	2.64 : 1	maternal	ns
M_2 -6	2.35 : 1	paternal	ns
M_2 -7	1.67 : 1	paternal	

When the results were pooled for each M_2 , departures from the F_2 occurred in both directions. However, where they could be tested, these shifts were not significant. When families were considered separately (Appendix B3), six had significantly more, and two significantly less, of the recessive paternal types; one family had no maternal types at all.

v. **non-naked : naked caryopsis (N : n)**

There were only two treatments in which families produced consistent segregation ratios for caryopsis type.

GP : 4082

F_2	2.40 : 1		
M_2 -3	2.60 : 1	maternal	
M_2 -4	2.07 : 1	paternal	
M_2 -5	2.13 : 1	paternal	ns
M_2 -6	2.66 : 1	maternal	ns
M_2 -7	1.67 : 1	paternal	

Pooled M_2 ratios hovered either side of that belonging to the F_2 . But when families were evaluated separately, six had shifted significantly towards the paternal compared to only one with an excess of maternal types.

- **linkage study : qualitative characters**

Only two of the genetic markers focused on in this study were located on the same chromosome : rough/smooth awns, and long/short rachilla hairs (See Figure 21). The F_2 segregation ratio for this combination of genes was tested against the 9:3:3:1 ratio expected in the case of independent inheritance to confirm linkage:-

RS : Rs : rS : rs

Observed 317 : 66 : 60 : 55

Expected 280.1 : 93.4 : 93.4 : 31.1

$$\chi^2 = 43.21$$

$$df = 3$$

$$P = ***$$

The phenotypic frequencies of M_2 families that departed significantly from the F_2 are given in Table 29, together with sources of variation. All of these families had fewer dominant maternal phenotypes, and all but one had more recessive paternal types.

For the most part, departures from the F_2 owed their origin to disturbed ratios for one or other of the genes involved. One 3Krad family proved the exception having individual gene frequencies that were consistent with those of the F_2 . Here the excess of paternal types seemed in part due to a reduction in the frequency of cross-overs between the two genes.

When all genes were considered together, 12 M_2 families had segregation ratios that differed significantly from that of the F_2 (Table 30). Once again, the source of much of this variation was the disturbance of the segregation ratios for individual genes. Recombinational differences in the linked pair of genes played a part as well. The variation not accounted for by these factors probably reflects the relatively small family size (maximum of 60 plants) in comparison to the 32 phenotypic classes.

The frequency of the maternal phenotype (RVOSN) in the F_2 was 0.228 (see Appendix B2). While only one of the 12 families had more of these maternal types, 11 had fewer and the mean frequency was appreciably lower at 0.111.

		PHENOTYPIC FREQUENCY				SOURCE OF VARIATION		
		RS	Rs	rS	rs	R:r	S:s	Remainder
F2		.637	.133	.120	.110			
M23	Krads							
	7	.600L	.080L	.080L	.240H	1.38	1.57	6.56
	13	.563L	.164H	.055L	.218H	0.63	5.66	2.38
M24	Krads							
	7	.520L	.100L	.140H	.240H	6.05	0.03	3.23
	pooled	.615L	.129L	.103L	.153L	2.14	4.47	1.78
M25	Krads							
	pooled	.533L	.208H	.108L	.150H	0.63	8.50	-0.10
M26	Krads							
	6	.119L	.000L	.643H	.238H	101.67	0.01	23.54
	7	.611L	.278H	.019L	.093L	4.42	5.66	3.28
M27	Krads							
	1	.559L	.176H	.000L	.265H	0.17	8.62	3.50
	4	.400L	.200H	.067L	.333H	0.04	8.82	0.12
	pooled	.517L	.233H	.050L	.200H	0.41	27.53	-2.97

H = higher than F₂, L = lower than F₂

Table 29: M₂ families with phenotypic frequencies significantly different from that of the F₂

chi²

	All Genes	R:r	S:s	V:v	O:o	N:n	RS:Rs:rS:rs
M ₂ -3 (2)	83.03	-	-	67.99	-	-	-
(3)	42.38	-	-	-	35.59	-	-
(7)	29.67	-	-	-	-	-	9.51
M ₂ -4 (1)	36.05	-	-	5.53	5.32	5.46	-
(2)	30.22	-	-	-	-	-	-
(10)	38.72	-	-	-	-	38.59	-
M ₂ -5 (1)	28.22	-	-	-	-	-	-
(2)	30.11	-	-	16.08	-	-	-
(3)	38.37	-	4.78	-	-	-	-
(pooled)	55.36	-	8.50	-	-	-	9.03
M ₂ -6 (6)	106.03	101.67	-	8.65	-	-	125.22
(7)	89.47	4.42	5.66	40.42	5.20	-	13.36
M ₂ -7 (3)	48.55	-	-	-	41.92	13.92	-
(pooled)	65.51	27.53	-	-	-	-	24.97

Table 30: M₂ families in which there were significant departures from the F₂ segregation ratio for all genes.

- linkage study : quantitative characters

In addition to the major gene analyses, the relationship between continuous characters was examined for the effects of the X-ray treatment. The investigation focused on four of the characters measured: height, tiller number, ear length and awn length.

The results of χ^2 tests for heterogeneity of the correlation coefficients carried out for each treatment are displayed in Table 31 (see also Appendix B4). The distributions of correlation coefficients are shown in Figures 38 to 43.

For all but one pair of variables, the F_2 was homogeneous. In the case of ear length/awn length, heterogeneity was significant at the 5% level making it impossible to produce a combined estimate of r with which to make comparisons between the F_2 and the M_2 . Significant heterogeneity also occurred in the 3 and 4 Krad treatment groups.

The correlation coefficients of the parents were generally low and positive (ranging from 0 to +0.7), with the F_2 distributions roughly centering on these values. The most noticeable feature of the M_2 was the downward extension of its distribution relative to the F_2 . In the case of height and ear length for example (Figure 39), the correlation coefficients were approximately +0.7 for the maternal parent, +0.5 for the paternal parent, -0.1 for the lowest F_2 family, but -0.9 for the lowest M_2 . So in this M_2 family, as height increased ear length tended to decrease with about 80% of the variability in one character linked to variability in the other.

This tendency was also marked in the case of ear length and awn length (Figure 43), where the correlation coefficients of the parents and F_2 families were all positive, but that of the lowest M_2 was -0.8.

When individual families were compared to the combined F_2 estimate of ' r ', 44% of them were found to differ significantly (see Appendix B4).

	ht & tn	ht & el	ht & al	tn & el	tn & al	el & al
F ₂	NS	NS	NS	NS	NS	*
M ₂ -3	***	NS	*	NS	NS	NS
-4	NS	***	NS	NS	NS	***
-5	NS	NS	NS	NS	NS	NS
-6	NS	NS	NS	NS	NS	NS
-7	NS	NS	NS	NS	NS	NS

Table 31: Results of χ^2 tests for heterogeneity of the correlation coefficients within each treatment.

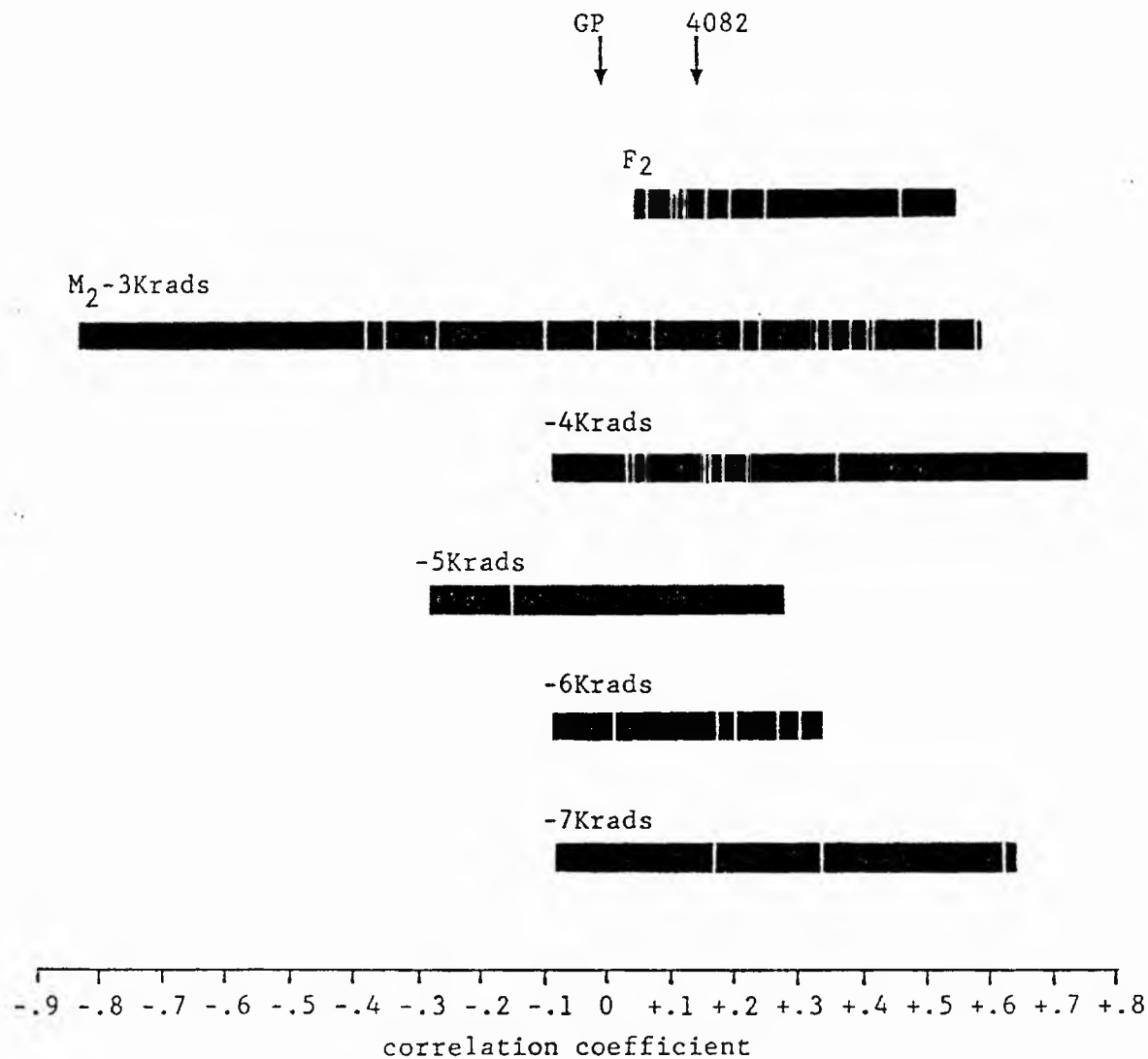


Figure 38 : Distribution of 'r' for height and tiller number

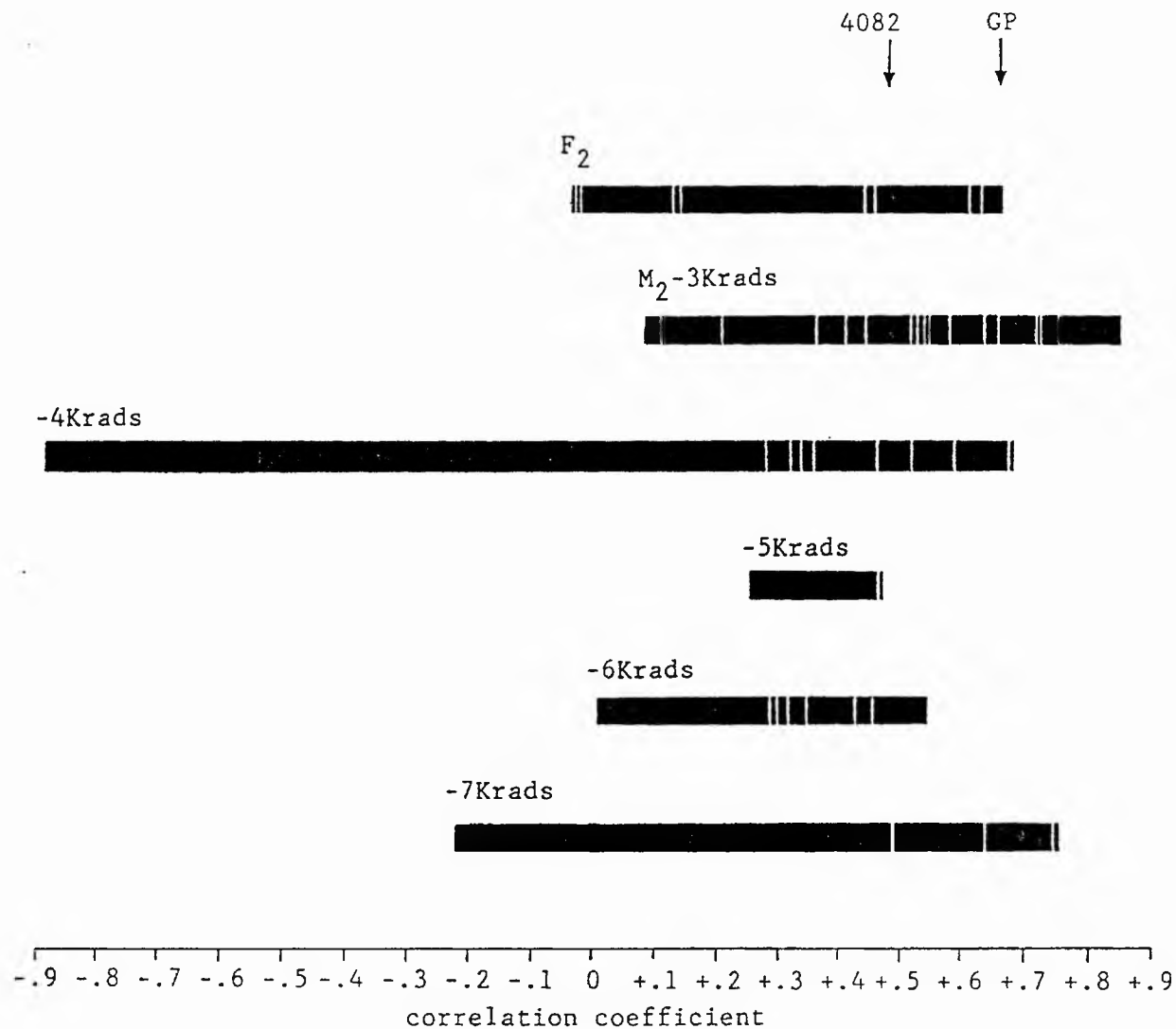


Figure 39 : Distribution of 'r' for height and ear length

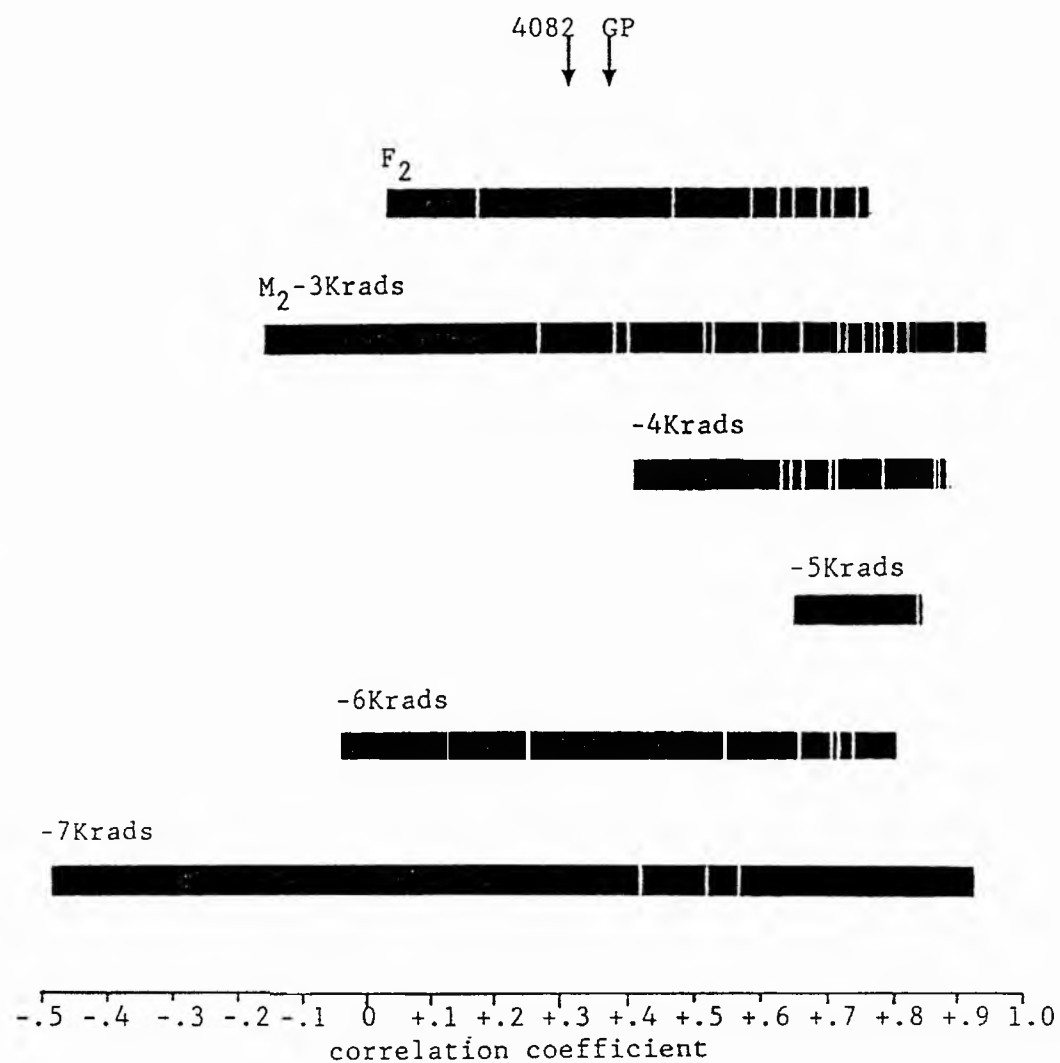


Figure 40 : Distribution of 'r' for height and awn length

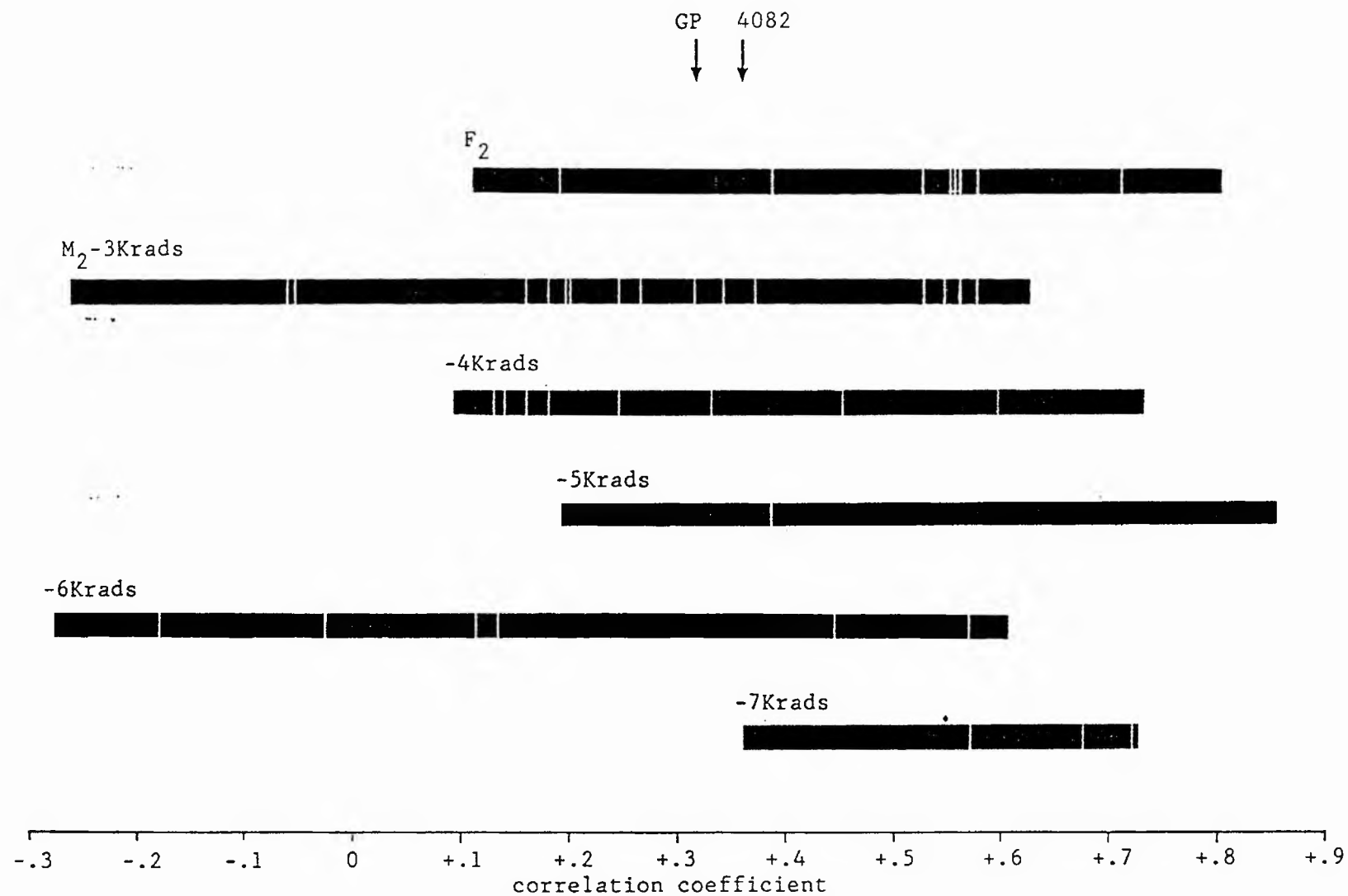


Figure 41 : Distribution of 'r' for tiller number and ear length

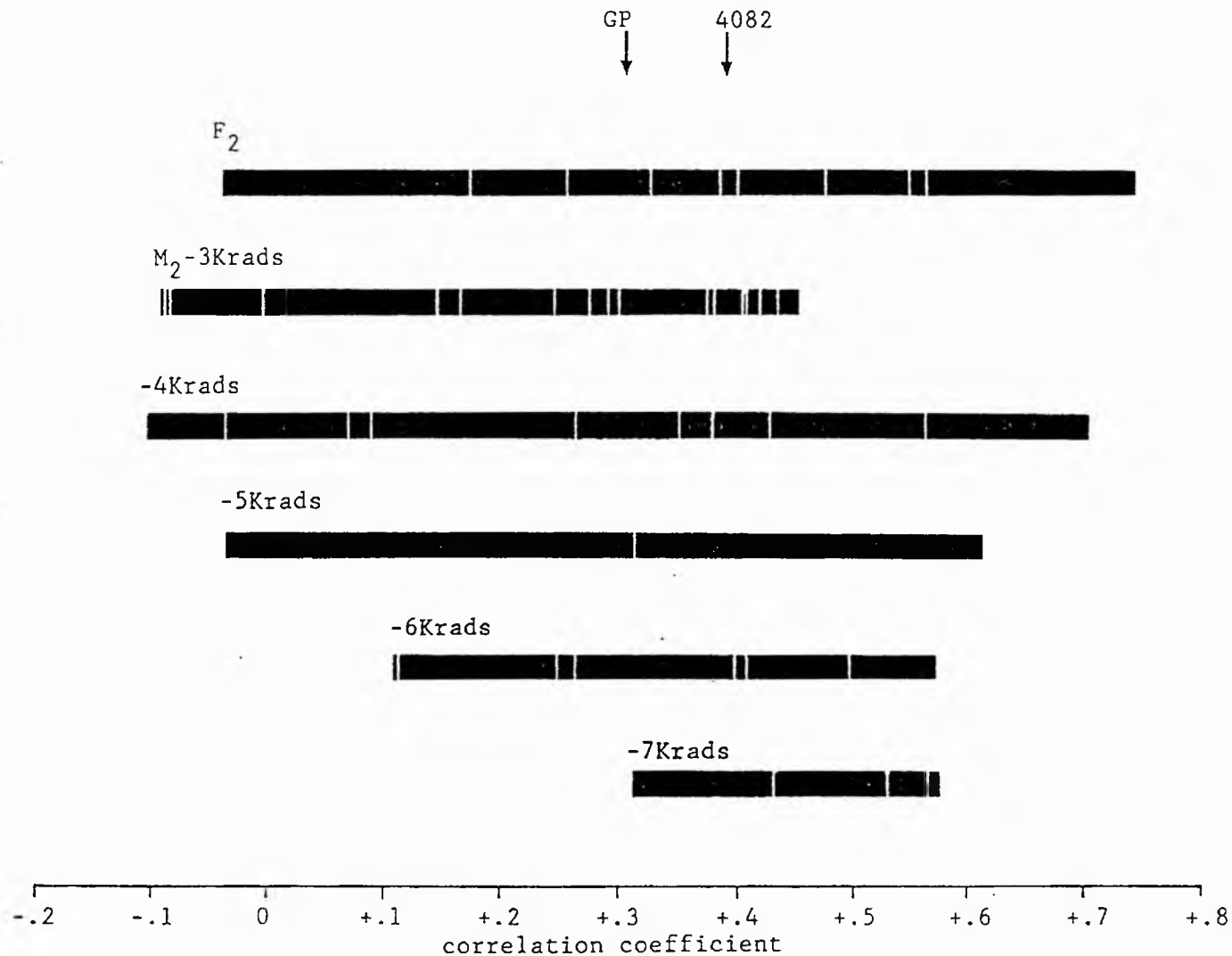


Figure 42 : Distribution of 'r' for tiller number and awn length

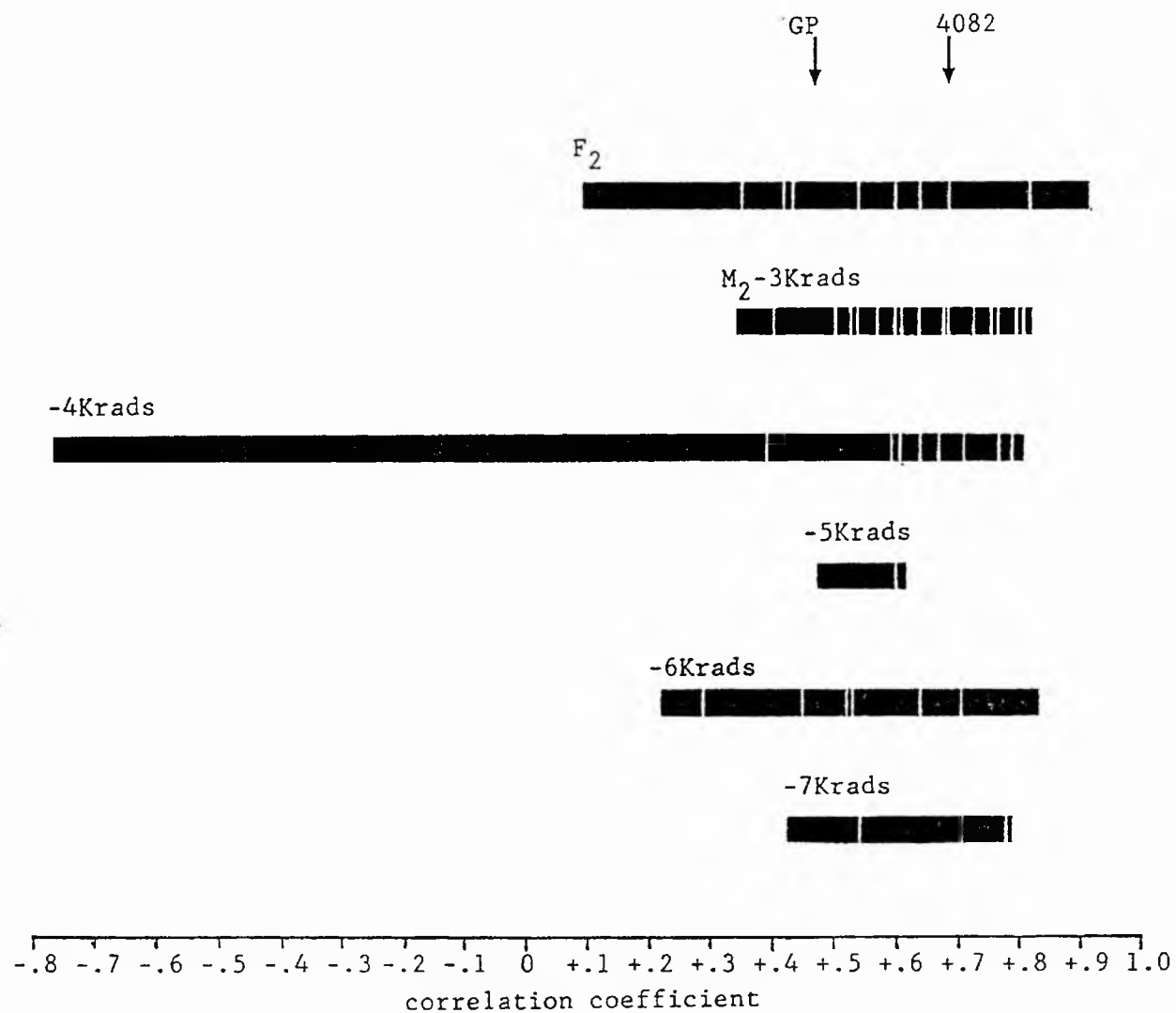


Figure 43 : Distribution of 'r' for ear length and awn length

Discussion

Since the egg transformation models of Pandey have largely been dismissed, the irradiated pollen debate has focused on whether preferential elimination of mutational damage, or its persistence, plays a greater part in determining M_2 phenotype. That is, the dispute is over what happens during the formation of the second generation rather than what occurs initially during fertilisation. So one might expect that similar mechanisms come into play regardless of which gamete is irradiated (although there are likely to be practical differences e.g. in dosage).

The first questions to be answered, therefore, were whether or not paternal trends could be induced in the second generation following crossing with irradiated ovules. And if so, was the technique any easier to carry out than pollen irradiation? In addition, it was hoped that some light would be shed on the mutational damage/genomic selection debate.

- the first generation

As in the irradiated pollen experiment, the M_1 was similar in appearance to the F_1 indicating a substantial amount of information had been transferred from the irradiated parent. Although the M_1 was slightly more variable than the F_1 , there were no grossly abnormal irradiated hybrids. And again, seed set was the only continuously varying character to fall with increasing dose. The relative uniformity makes it unlikely that selection would be successful in the first generation. So from this point of view, ovule irradiation has no advantage over its male equivalent.

- quantitative characters

In the irradiated pollen study, reciprocal crosses were performed in an effort to establish the relative importance of mutational damage and genomic selection. Even though irradiated ovule crosses were made in only one direction, the differences between the F_2 and the paternal parent were such that for different characters an increase in score, a decrease in score, and no change in score relative to the F_2 would have been expected had genomic selection predominated. If, on the other hand, mutational damage had been most important, consistent decreases in M_2 score would have occurred in all these cases. In fact, a downward shift from the F_2 didn't occur for any character.

In the case of height, the F_2 was taller than GP but shorter than the paternal parent, 4082. While the means for all M_2 treatment groups were indeed larger than that of the F_2 , a dose dependent trend was not readily apparent. Interestingly, when the distribution of family means was examined, it was only the lowest dose M_2 that had a range which included that of 4082.

As a measure of fertility, tiller number would be expected to be particularly susceptible to radiation damage. The F_2 produced more tillers than the paternal parent and an equivalent number to the other parent. Surprisingly then, where there was a shift in the M_2 , it was for increased tiller number - a result which runs counter to that expected for either of the proposed mechanisms.

Ear length was another character for which a decrease in the M_2 would have been consistent with a paternal shift. This time, all M_2 treatment groups had a mean ear length that was equivalent to that of the F_2 (although the two lowest dose treatments did have families with mean ear lengths which were as low as that of the paternal parent).

The last quantitative character to be considered was awn length, the average for the F_2 being equal to that of the paternal parent and larger than that of GP. If genomic selection were key, no shift would be apparent in the M_2 which is exactly what happened in all but the 7 Krad treatment group (this had longer awns).

To summarise, while a paternal shift wasn't always apparent in the M_2 , no character suffered a reduction in vigour following the radiation treatment either. So it's unlikely that widespread mutational damage persisted to the second generation. When the distributions of family means were examined, it was the lowest dose treatments that produced families most like the paternal parent. It was also in these treatments that the most variable M_2 families were found. This may be beneficial from the practical point of view, because the lower the dose needed to produce the desired effect the less effort required.

While there were no significant differences between F_2 families for quantitative characters, such differences were observed, although infrequently, in the M_2 . Rather than an indication that parts of the genome had been preferentially affected, the lack of much significance is probably a reflection of the relatively small numbers of degrees of freedom in some of the groups.

- qualitative characters

While the results of the major gene analysis in the pollen study were surprising (an excess of paternal types occurring almost as often as an excess of maternal types), those of the ovule study were as expected.

The pooled segregation ratios are set out in the following table. There were no non-parental types.

	R:r	S:s	V:v	O:o	N:n
GP	1:0	1:0	1:0	1:0	1:0
4082	0:1	0:1	0:1	0:1	0:1
F ₂	3.40:1	3.10:1	3.24:1	2.38:1	2.40:1
M ₂	3.13:1	2.51:1	2.28:1	2.16:1	2.38:1

Deviations from the F₂ were generally towards the paternal. In the case of caryopsis type (N:n), the excess was not that great in the pooled M₂. But when families were considered separately, six had a significant excess of plants with the paternal naked caryopsis whereas only one had significantly more maternal types. Overall, 86% of the cases where families differed significantly from the F₂ had an excess of paternal phenotypes.

Since the irradiated parent was also the dominant parent for those major genes under consideration, proponents of the radiation damage theory might argue these results reflect impaired expression of the maternal alleles present. However, some non-parental phenotypes might also have been expected if this were the case. And the absence of a trend for reduced vigour in the M₂ also makes radiation impairment a less plausible argument.

- linkage

Because only two of the genes examined in this study were located on the same chromosome, discussion of the effects of ovule irradiation on major gene linkage is limited. When the M₂ segregation ratios for this pair of genes were examined, virtually all of the departures from the F₂ could be explained by a disturbed segregation pattern for one and/or other of the genes involved.

As far as linkage between continuously varying characters was concerned, the striking feature of the M_2 was the existence of families with high, but negative, correlation coefficients. That is to say, there were cases where there was an association between a high score for one character and a low score for the other. Had mutational damage been widespread, low scores would have tended to have been associated with other low scores instead.

- conclusion

The importance of gamete irradiation as a technique in plant breeding will depend on the degree to which information from the irradiated parent is transferred from the first generation. Neither the ovule nor the pollen experiment have produced any evidence to support the contention that considerable radiation induced damage persists beyond this generation. And in the ovule study, at least, significant shifts towards the unirradiated parent in the second generation did occur.

Not only was ovule irradiation the more successful of the two techniques, it was also easier to perform. Even though whole plants had to be transported for irradiation purposes (as opposed to detached ears), and an X-ray machine had to be employed to deliver the dose, these factors were more than balanced by the higher seed set and better germination.

Without resorting to embryo rescue, the highest dose at which resulting seeds were successfully germinated was 8 Krads. It may be that little is gained from using even higher doses since surviving M_1 plants will probably be those that are least damaged and perhaps like their lower dose counterparts anyway.

A comparison of M_2 and F_2 results is presented in Table 32. Although the frequency of significant departures from the F_2 appears to be marginally greater in the higher dose groups, it was among the lower doses that families were most like the paternal parent for quantitative characters (see histograms of family means). As there was no association between a disturbed linkage pattern and/or segregation ratio and a lower quantitative score, the persistence of considerable radiation induced damage again seems unlikely.

	qualitative traits ^a	quantitative traits ^b	linkage ^c
M_2 -3KradS			
family 1	-	+-	--
2	*	---	-*
3	*	---	-*
4	-	+-	--
5	*	+-	-*
6	-	+++	-*
7	-	+++	**
8	*	+++	--
9	*	+-	--
10	*	---	--
11	-	+-	-*
12	*	++-	-*
13	*	+++	**
14	-	+-+	-*
15	-	+-+	--
16	-	+++	--
17	-	---+	--

KEY

a * = significant departure from the F_2 for a major gene segregation ratio

b = comparison with F_2 height, tiller number & ear length

c * = pattern of linkage significantly different to that of the F_2 -qualitative and quantitative characters

Table 32: Comparison of M_2 with F_2 results

	qualitative traits ^a	quantitative traits ^b	linkage ^c
M_2 -4KradS			
family 1	*	+--	--
2	-	++-	--
3	-	+--	--
4	*	---+	-*
5	-	---	-*
6	-	---	--
7	*	---	*-
8	*	+--	-*
9	*	++-	--
10	*	++-	--
M_2 -5KradS			
family 1	-	---	--
2	*	+--	-*
3	*	+++	--
M_2 -6KradS			
family 1	-	+++	-*
2	*	+--	--
3	*	---	--
4	-	++-	--
5	-	+--	-*
6	*	-++	*-
7	*	+--	**
8	-	++-	--
M_2 -7KradS			
family 1	*	+++	**
2	*	---+	--
3	*	-+-	--
4	*	+++	**
5	*	++-	--

Table 32 Continued

While the results of this experiment suggest that ovule irradiation is promising, more studies are needed to confirm the technique's potential. It may be that it is most successful in diploid species like barley, and it may also be technically more difficult to perform in other crops. Nevertheless, as an addition to a conventional backcrossing programme, it would require little more than an excursion to irradiate plants. With the potential to cut down a lengthy and laborious backcrossing schedule, perhaps that would be a trip worth making.

PART 4

IRRADIATED POLLEN CROSSES IN THE POTATO

Introduction

Experimental Design

Method

- crossing procedure
- handling seed
- handling tubers
- multiplication
- pcn resistance tests
- virus resistance tests
- cytology
- the glasshouse experiment

Results

- cytology
- pcn resistance
- PVX resistance
- establishment
- height
- tuber number
- tuber weight
- stem colour
- tuber colour

Discussion

Introduction

When the Spaniards arrived in South America in the sixteenth century, the tetraploid potato, Solanum tuberosum, had already been domesticated and dispersed over a considerable area. Although its wild origin has long since been obscured by millenia of cultivation, the domesticated potato has many wild relatives within the genus Solanum. Of the more than 2,000 species, approximately 150 are tuber-bearing [Plaisted, 1980] and about 70% are diploid [Hawkes, 1958]. Wild potatoes are of more than purely botanical interest because they may considerably augment the gene pool available to the breeder.

While the primitive cultivars of the Andes were not bred in the true sense of the word, selection for higher yields presumably took place even if unconsciously. The artificial hybridisation of potatoes, however, probably did not take place until the species had become established in Europe.

The potatoes introduced from South America are believed to have been samples of S. tuberosum subsp. andigena from Colombia. That being the case, introductions would have been short-day types in terms of induction of tuberisation. Under the long-day conditions of northern Europe, these potatoes would have formed tubers late and given a poor yield. So selection of seedlings derived from true seed must have taken place before the potato could become widely established in the region. [Burton, 1989].

According to Davidson [1935], the first known reference to distinct varieties in the UK was in 1730 when five types of potato were recognised. As early as 1807, Knight was investigating the cross-pollination of these varieties [Glendinning, 1983]. So, by the early 19th Century, the two essential methods of conventional plant breeding had been attempted in the potato.

To begin with, the desire for new varieties arose because of the need to replace cultivars that had "degenerated" during years of vegetative reproduction. Later, a major stimulus to potato breeding occurred when, between 1845 and 1847, severe epidemics of blight (Phytophthora infestans) swept across America and Europe [Stuart, 1937]. Interest grew with the alarming spread of wart disease (Synchytrium endobioticum), when not only were immune clones identified but immunity was also recognised as a heritable trait [Mackay 1987]. Today, disease resistance remains the impetus for many potato breeding programmes.

Because potatoes are normally propagated by vegetative rather than sexual means, their breeding is easier in one important aspect. That is, as long as vegetative reproduction is followed (and providing mutations do not occur), any selection will keep true-to-type. So new varieties may be selected as F_1 plants. Similarly, novel techniques are relatively easy to perform because the problem of coming true-to-type after sexual reproduction is avoided [Howard, 1978]. That said, the rate of increase by means of tubers is much slower than that by seed so the process of multiplying up to a marketable quantity is far from rapid.

Despite the potato's suitability for experimental techniques, most, if not all, of the cultivars currently available are the product of traditional breeding at the tetraploid level [Mackay, 1987]. Parental clones are crossed and the breeder screens resulting progenies for recombinants which combine the best features of both parents. Since detailed knowledge of the heritability of many characters of commercial significance is lacking, parents are generally selected on their own performance in the hope that their better properties will be passed on. Howard [1970] has estimated that the chance of a seedling produced by this method becoming a useful variety is as low as 1 in 10,000. Moreover, the realisation of desirable crosses is often hampered by pollen sterility or bud abscission [Burton, 1989]. Not surprisingly, alternative approaches to potato breeding have received considerable attention.

One such approach involves the use of 'dihaploids'. Although there are cultivated diploid potatoes in South America, the tetraploid S. tuberosum is by far the widest grown species. Hougas and Peloquin [1958] found that if S. tuberosum clones were pollinated with certain clones of S. phureja, a proportion of the progeny were parthenogenetic haploids (with the diploid number of chromosomes). In order to distinguish these plants from those which were genuinely diploid, the term 'dihaploid' was introduced. Although primary dihaploids are weak, they can produce vigorous offspring when crossed with either diploids (cultivated or wild), or with tetraploid varieties (when, because of the high proportion of unreduced gametes produced by dihaploids, the progeny are largely tetraploid). Alternatively, the chromosome number of dihaploids with desirable genes may be doubled with colchicine prior to their inclusion in a breeding programme.

Opinion varies as to the potential value of dihaploids in potato breeding. While Peloquin [1983] described them as a means of capturing the genetic diversity present in Solanum species, Hermesen [1983] noted the drawbacks resulting from their narrow genetic base. More recently, the production of monoploid potatoes has also received attention [Mackay, 1987].

Another approach relies on the assumption that S. tuberosum subsp. andigena material originally brought over to Europe was of limited amount. So the initial selection for long-day tuberisation types must have been from a very restricted genetic base. It ought to be possible to rectify this situation by incorporating varieties of subsp. andigena into modern breeding schedules. However, in order to eliminate the late tuber initiation, several generations of backcrossing would have to be undertaken.

To overcome this problem, Simmonds [1961, 1966, 1969] sought to 'recreate' subsp. tuberosum on a broad genetic base by growing a wide range of Andean potatoes in England, and mass selecting over a period of years. Clones from the resulting 'Neo-Tuberosum' population have since been successfully crossed with their tuberosum relatives [Mackay, 1987].

Apart from being vegetative by nature, the potato is also amenable to a variety of tissue culture techniques. This has led to the rapid uptake of the species as a vehicle for genetic manipulation research.

'Somaclones', for example, may be produced by regenerating plants from callus derived from explants including protoplasts. Those produced from the same cultivar may be highly variable. Even somaclones obtained from callus derived from the same protoplast can vary [Thomas et al., 1982]. Importantly, these variant characters are retained, at least for several generations [Secor and Shepard, 1981]. Attempts are underway to use somaclonal variation to improve deficiencies in existing cultivars [reported by Mackay, 1987]. However, some authorities believe somaclones merely increase the frequency of variants that can occur spontaneously anyway and so are of limited value [Sanford et al., 1984].

Isolated protoplasts have also been fused, cultured, and regenerated to form 'somatic hybrids'. This technique may be valuable in hybridising diploids in which sterility is a problem. Alternatively, it may enable the utilisation of wild Solanum species that are incompatible with Solanum cultivars.

Another possible use for isolated protoplasts is the in vitro selection of disease resistance. This may be achieved by exposing cultures to pathogens, or to the toxins produced by them. Behnke [1980] achieved some success with this method in selecting for resistance to late blight.

None of the techniques so far mentioned involve limited or single gene transfer from one clone to another. Since many clones are extremely heterozygous, when they are crossed their good qualities may be transmitted to only a very small proportion of their progeny. So a technique that permitted the introduction of a specific gene into a genotype without involving sexual reproduction would be especially useful in the potato.

As the potato is one of the crops that is readily infected by Agrobacterium tumefaciens, the ability to insert genes into potatoes using this organism is being established [Flavell, 1987]. It also seems that there are unlikely to be difficulties in transferring DNA into potato protoplasts by direct DNA uptake procedures [Shillito et al., 1985]. However, the efficiency of regenerating transformed plants from these cells needs to be improved. And even then, the activity of inserted genes may vary from plant to plant.

Obviously, genes must also be identified before they can be inserted. And, as yet, methods for deleting or replacing genes have not been developed [Flavell, 1987]. So, there is still some way to go before genetic engineering techniques will become routinely available to the potato breeder.

By comparison, pollen irradiation (if effective) would be a relatively simple and cheap means of facilitating limited gene transfer. Because sexual reproduction is an essential feature of the technique, selection would still form part of the procedure. Nevertheless, the time taken to achieve limited gene transfer may be substantially less than that required using conventional means.

To find out if the potential of this technique that had been identified in other plant species could be realised in the potato, an experiment was set up in the summer of 1981.

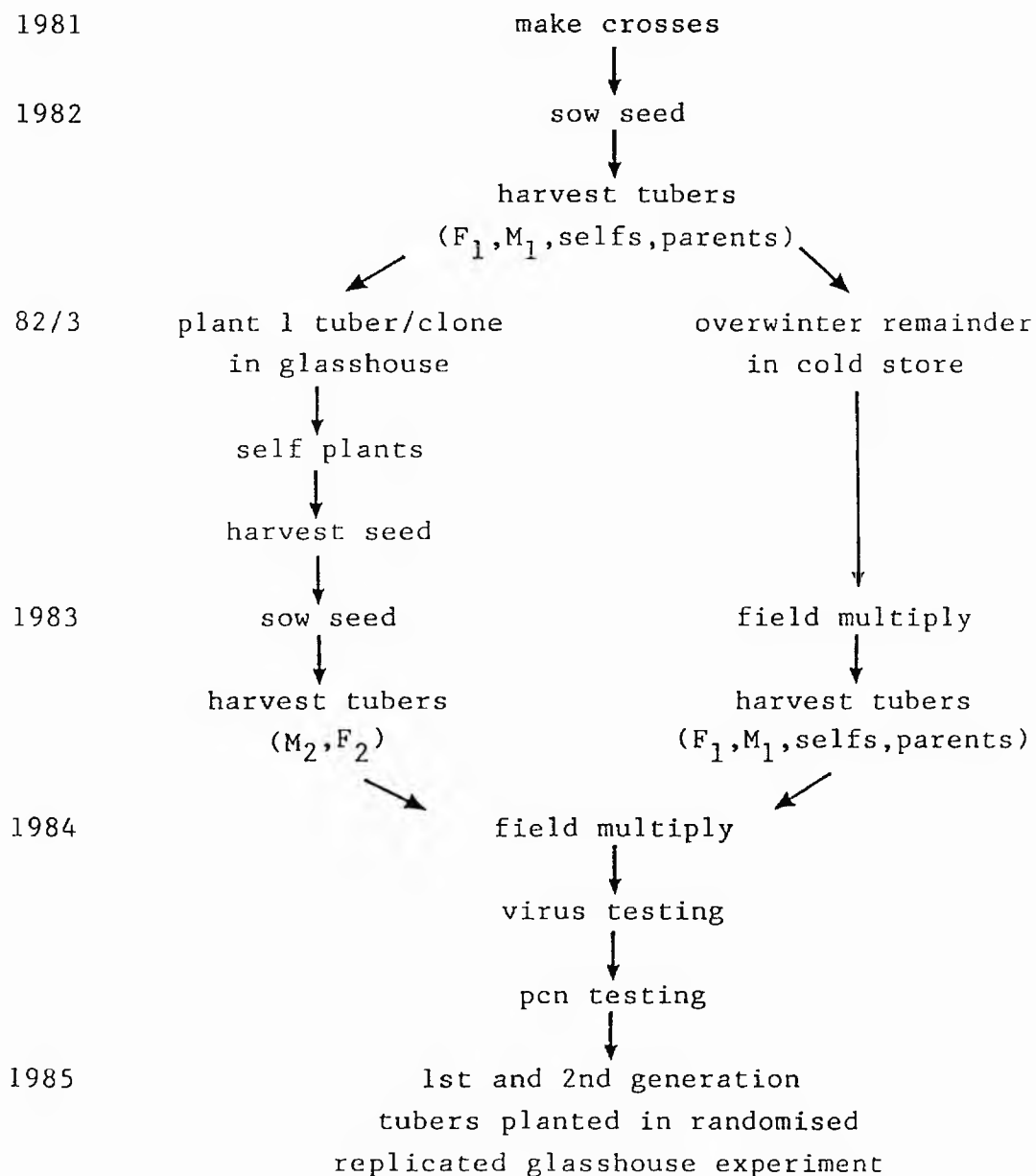
Experimental Design

Three commercial potato varieties: Pentland Ivory (PI),

Desiree (D) and Cara (C) were used in this experiment. Irradiated and control crosses were made at the doses (Krad) scheduled below.

		paternal parent		
		D	PI	C
maternal parent	D	0	0,5,10,15,20	0,5,10,15,20
	PI	-	0	0,5,10,15,20
	C	-	-	0

The method by which first and second generation material was obtained is outlined in the following diagram.



The cycle from selfing M₁ plants to producing M₂ tubers was repeated in 1984 to create more M₂ families. The study culminated in a glasshouse experiment in which the tuber-grown F₁, F₂, M₁, M₂, parents and selfs were compared. Each hybrid clone appeared once in each of two randomised complete blocks.

Method

- crossing procedure

The three parental cultivars were grown in the glasshouse during the early summer of 1981. Emasculation took place at the mature bud stage, just as the petals appeared ready to separate. The five anthers could then easily be removed with forceps after opening the petals.

The pollen to be irradiated was collected within a couple of days of the corolla opening by inserting a blunt instrument at the base of the suture in the anther lobe and scraping it the length of the anther. Cross contamination was avoided by cleaning the scraper with alcohol. After collection in gelatin capsules, pollen was transported to the Western General Hospital, Edinburgh, where it was subjected to gamma radiation doses of between 0 and 20 Krads. As soon as possible after treatment, it was applied to the stigmata of newly opened, emasculated flowers which were labelled with the crossing details.

The procedure for selfing differed from the above in that flowers were not emasculated, and pollen removed from anthers was applied directly to the stigmas of open flowers on the same plant.

- handling seed

In order to avoid seed losses, fruits were inserted into bags as they developed. These were then secured around the stem. When the berries began to soften (about 1 1/2 to 2 months after pollination), they were removed from the plant, stored if necessary and the seed extracted.

Ripe fruits were cut and placed in a blender (with the blades reversed). After blending with water for a short time, the seeds (which sank to the bottom) could be separated from the pulp (which was decanted off the top). Seeds were then rinsed, strained and laid out to dry on paper towels for 2-3 days with their labels. They were collected in envelopes and dried further in a desiccator. Although most newly harvested potato seeds are dormant, this dormancy generally disappears after a few months in storage [Plaisted, 1980].

Seeds were sown in Petri dishes kept at 20 °C until germination. Seedlings were transferred to Jiffy 7s and then potted on into 10cm plastic pots containing Levington Universal Compost (75% peat, 25% sand). Plants were raised on sand beds in the glasshouse where the minimum daytime temperature was 15 °C and that at night was 5 °C. When necessary, plants were fed with Phostrogen and the glasshouse was routinely fumigated with Pirimor. When plants were raised over the summer months, tubers were ready for harvesting 3-4 months after planting. When grown over winter under lights (maintaining a 16 hour day length), harvesting occurred after about 5 months.

- **handling tubers**

When tubers were ready for harvest, the watering system was switched off, top growth was cut-off and the pots were allowed to dry out. The contents of each pot were tipped out in turn and the tubers were separated. They were then scored and/or allocated to paper bags to be stored for future use e.g. flowering, multiplication, pcn testing, virus testing or cytology. Bags were then kept at 3 °C in a cold storage room until needed.

- **multiplication**

The single largest tuber produced by each clone was planted at the Blythbank high grade seed farm, Peeblesshire, to be multiplied for use in later stages of the experiment (see experimental design).

- **pcn resistance tests**

Two species of cyst nematode infest potatoes in Northern Europe: Globodera rostochiensis (the golden cyst nematode) and G. pallida (the white cyst nematode) [Stone, 1972]. Within each species there is a range of pathotypes [Kort et al., 1977]. Affecting crops throughout the UK, these soil borne pests can cause considerable damage to potato roots resulting in a dramatic reduction in yield. There are a number of effective nematicides, but their application obviously adds to the cost of potato production.

Breeding for resistance began after Ellenby [1952] discovered sources of resistance in a number of accessions in the Commonwealth Potato Collection. The first source to be exploited was derived from the primitive cultivated potato S. tuberosum subsp. andigena. It was controlled by a single gene, H_1 , which was soon incorporated into cultivated subsp. tuberosum clones. However, by 1957 a population of nematodes had been discovered which could produce many cysts on the roots of even those potatoes carrying the H_1 gene [Dunnett, 1957].

Subsequently, it was found that H_1 is effective against pathotypes Ro1 and Ro4 of G. rostochiensis, but not against G. pallida. Fortunately other sources of resistance have been found in the diploid S. vernei and some clones of subsp. andigena. Both these sources are inherited quantitatively.

As far as the cultivars in this experiment are concerned, Cara possesses the H_1 gene, while Pentland Ivory and Desiree are both susceptible varieties. The progenies of the PIxC and the DxC crosses were therefore screened for resistance to the golden cyst nematode.

Tubers were placed in 60ml 'Clearpac' containers partly filled with John Innes No. 2 compost which had been previously sterilised by heating to a temperature of 160°C [Phillips et al., 1980]. After planting, containers were inoculated with an egg suspension of the Ro1 pathotype of G.rostochiensis. The concentration was adjusted to give 20 eggs/g dried soil and a moisture level of 30%. Immediately after inoculation, canisters were sealed and incubated in the dark at a temperature of 20°C for 7 weeks [Phillips et al., 1980].

Following accepted methods of assessment, the number of developing females visible through the walls of each container was then counted. Plants with many cysts were declared susceptible, while those with none or very few were pronounced resistant.

virus resistance tests

Several types of virus resistance can be distinguished in potatoes: tolerance (where there are no obvious symptoms and yield losses are small), infection resistance (where only a small percentage of plants become infected), hypersensitivity (usually giving field immunity) and extreme resistance (or immunity) [Howard, 1978]. While the first two types are usually under polygenic control, the last two are often due to single dominant genes. Single gene resistance to most viruses is widespread, being found in *Tuberosum* and *Andigena* potatoes, in cultivated diploids and also in some wild species.

The virus used in this study was potato virus X which is spread by leaf contact and exists as many strains. As with *G. rostochiensis*, Pentland Ivory and Desiree are susceptible to virus X, while Cara possesses the N_x gene making it field immune. Although mild strains of the virus may produce negligible symptoms and only very small reductions in yield, co-infection with virus A causes 'crinkle' which can result in considerable losses.

Clones from the Desiree x Cara group of crosses were screened for resistance to PVX. Healthy plants were sap-inoculated with Muir's Common Field X in 1984.

Where possible opposite leaves were chosen for inoculation. These were marked by removing a small disc of tissue and then dusted with carborundum powder. Inoculation was achieved by taking infected material and grinding it with phosphate buffer using a sterile pestle and mortar. The expressed sap was gently applied to leaves with the forefinger, stroking away from the petiole towards the tip. The inoculum was then washed off the test plants using a jet from a water bottle. Plants were examined for responses to inoculation at approximately weekly intervals.

Susceptible plants generally displayed mosaic symptoms on the leaves. Local necrotic lesions or halo necrosis (fine necrotic spots in larger chlorotic spots) were signs of resistance unless mosaic symptoms were also evident. Plants with no symptoms were either resistant to PVX or escapes.

To confirm the results, tubers from plants which did not display symptoms were grown-on in 1985. Unless they already showed symptoms, these plants were again inoculated and their response re-examined.

- cytology

A limited examination of F_1 and M_1 mitosis was made for all groups of crosses. Sampled roots were pre-treated in 8-hydroxyquinoline for 3 hours at 18 °C, and then hydrolysed in 1N HCl at 60 °C for 10 minutes. The root tip was tapped out in 45% acetic acid and stained in 1% crystal violet.

- the glasshouse experiment

The study culminated in a randomised, replicated glasshouse experiment in 1985. The F_1 , F_2 , M_1 , M_2 , selfs and parents were grown from tubers so that they could be compared for a number of characters. Similar sized tubers were planted in Levington Universal Compost in 10cm plastic pots. Apart from the parents, each clone appeared only once in each of two reps. There were 10 pots to each row. The characters measured were:-

i. establishment

When field grown, the rate at which potatoes make early top growth and give good cover of the ground is important in terms of weed control. Moreover, a rapid early growth of tops also appears to be a prerequisite for high yields, especially in early potatoes [Howard, 1977].

Although glasshouse behaviour may not closely follow the pattern of that in the field, it does enable a comparison of different treatments to be made. So 18 days after planting, each pot was examined for emerging shoots. Those which bore leaves were declared established.

ii. height

Because plants develop and die back at different rates, final heights could not be recorded. Instead, the height of each plant was measured 32 days after planting.

iii. colour in stem

At the same time as height was measured, the amount of red pigment present in the stem was recorded. There were four categories: none (1), little (3), some (6), much (9). Plants scoring 1 had completely green stems, while those scoring 9 were purple-stemmed.

iv. tuber colour

Tuber colour may be of commercial importance if it helps consumers to recognise a good quality variety. The three parents in this experiment have different tuber colours: Pentland Ivory is white, Desiree is red and Cara is parti-coloured red. As in the case of stem colour, four colour categories were distinguished. Pentland Ivory was at one end (no red), while Desiree was at the other (completely red).

v. tuber number &

vi. tuber weight

From the commercial point of view, it isn't usually total yield alone that is important, or total tuber number, but rather the yield of saleable tubers (ie those that fall with the acceptable size grades). Although growing small tubers in small pots is unlikely to provide a very accurate indication of field performance, tuber number and tuber weight were suitable characters with which to assess the effects of the radiation treatments.

Results

Although seed was produced at each of the selected radiation doses, in all but the 5 Krad treatment it was in very limited amounts. However, the conversion rate of seeds into plants was reasonably good:

	PIxC		DxC		DxPI	
	seed	plants	seed	plants	seed	plants
5Krads	80	56	32	26	34	19
10Krads	1	1	0	0	2	2
15Krads	5	4	0	0	8	7
20Krads	4	4	1	1	0	0

(Crosses were later repeated to increase the number of M_1 plants).

Of the few M_1 -20 Krad plants generated, none were successfully selfed. The number of second generation families raised for each other treatment is given below:-

	PIxC	DxC	DxPI
F_2 -0Krads	13	7	12
M_2 -5Krads	2	2	3
M_2 -10Krads	0	1	0
M_2 -15Krads	3	0	0

Up to 25 clones/family were grown in the final glasshouse experiment.

- cytology

While all the parents that were tested had the expected number of chromosomes ($2n=4x=48$), one of the 15 parental selfs examined was aneuploid as were two of the 19 F_1 plants (Table 33). The frequency of aneuploidy rose in the M_1 with 7 of the 22 plants having 46, 47 or 49 chromosomes [Borrino *et al.*, 1985].

number of chromosomes

	46	47	48	49
PI	-	-	3	-
D	-	-	3	-
C	-	-	3	-
PIxPI	-	1	13	-
DxD	-	-	-	1
F ₁ PIxC	-	1	11	-
M ₁ PIxC-5	2	-	5	-
M ₁ PIxC-15	-	-	6	-
F ₁ DxC	-	-	3	-
M ₁ DxC-5	1	-	2	-
M ₁ DxC-20				1
F ₁ DxPI	-	-	4	-
M ₁ DxPI-5	1	1	1	-
M ₁ DxPI-15	-		1	-
M ₁ DxPI-20	-	1	-	-

Table 33: Chromosome counts in first generation plants

pcn resistance

Summaries of the pcn resistance test results are presented in Tables 34 and 35. The findings confirmed the suspected dominance relationships. That is, Cara was the only parent to carry the dominant H_1 gene for resistance. The frequencies of resistant clones in the F_1 s (48%, 61%), and the F_2 s (35%, 41%), were consistent with the 50% and 37.5% expected from a simplex x nulliplex cross. Likewise, 74% of the Cara selfs were resistant which is very close to the 75% expected.

Had substantial elimination of the paternal genome occurred during the formation of the M_2 , this generation would have been expected to be more like the maternal self than the F_2 in expression. Whereas, if the persistence of radiation induced damage had been widespread, the difference between the M_2 and the F_2 may have been similar to that between the M_1 and the F_1 .

In fact, the M_1 -5Krad results were very similar to those of the F_1 . And although there were no resistant clones at higher doses, the sample size was small. When the results for all doses were pooled, the differences between the F_1 and M_1 were not significant for either the PIxC cross ($\chi^2 = 1.97$, $df = 1$) or the Dx C cross ($\chi^2 = 2.05$, $df = 1$).

The difference between the pooled M_2 and F_2 were, on the other hand, highly significant for both crosses ($\chi^2 = 19.53^{***}$ and 18.90^{***} for PIxC and Dx C respectively). In each case there were far fewer of the paternal-type resistant clones in the M_2 .

Two M_2 PIxC-15Krad clones gave resistant responses despite the fact the M_1 s they were derived from were susceptible (Table 34). As the M_2 s in question were only tested once, while the M_1 s were tested twice, it's possible the M_2 s gave a false negative response. Alternatively, mutation may have been responsible for the anomaly. But since there were two supposedly resistant clones, and as resistance is the dominant condition, this explanation is rather unlikely.

	number of clones	percentage resistant
PI		0%
C		100%
PIxPI	46	0%
CxC	57	74%
F ₁ PIxC	29	48%
M ₁ PIxC-5	13	46%
M ₁ PIxC-15	8	0%
F ₂ PIxC	185	35%
M ₂ PIxC-5	20	0%
M ₂ PIxC-15	33	6%

Table 34: Results of pcn resistance tests in the
Pentland Ivory x Cara cross

	number of clones	percentage resistant
D		0%
C		100%
DxD	31	0%
CxC	57	74%
F ₁ DxC	18	61%
M ₁ DxC-5	12	42%
M ₁ DxC-15	2	0%
F ₂ DxC	141	41%
M ₂ DxC-5	37	5%
M ₂ DxC-10	4	0%

Table 35: Results of pcn resistance tests in the
Desiree x Cara cross

PVX resistance

As expected, Desiree reacted with the susceptible response to potato virus X, while Cara displayed those symptoms associated with resistance (Table 36). The results of tests on the F_1 , F_2 and paternal selfs were consistent with Cara carrying a single dominant resistance gene in the simplex state. But the results for the irradiated generations were far from those expected.

Whether genomic selection or radiation damage had been the dominant mechanism, there should have been fewer of the paternal-type resistant clones in the M_2 and possibly less in the M_1 as well. Instead, there were more. However, there were only 4 M_1 plants and over 80% of the M_2 clones were derived from just one of these individuals.

establishment

The percentages of plants already established 18 days after tubers were planted are presented in Tables 37, 38 and 39. The results for the second generation have been split into two because there appeared to be a degree of dormancy in those tubers from the newer families which had been glasshouse grown. No such problem was apparent in the field harvested tubers. The new families have, therefore, been excluded from the discussion of the results that follows.

In the first cross (Pentland Ivory x Cara, Table 37), F_1 plants emerged before those of the M_1 (5 Krad $\chi^2 = 34.53^{***}$ df = 1, 15 Krad $\chi^2 = 17.22^{***}$). The M_1 's later emergence may have been a deleterious effect of radiation damage. Because the parental cultivars were heterozygous, if preferential elimination of the paternal genome had occurred between the first and second generations, the M_2 shift from the F_2 would have been towards the maternal self. In this case, the difference between the F_2 and the maternal self was not significant, so the M_2 results should have been indistinguishable from those of the F_2 as well. While this was the case for the 5 Krad treatment ($\chi^2 = 1.46$), at 15 Krads plants were significantly slower to emerge than their non-irradiated counterparts ($\chi^2 = 16.26^{***}$).

	number of clones	susceptible	percentage resistant	unknown
D	3	67	-	33
PI	3	-	100	-
DxD	17	100	-	-
CxC	18	22	78	-
F ₁ DxC	9	33	67	-
M ₁ DxC-5	4	-	100	-
F ₂ DxC	69	69	25	6
M ₂ DxC-5	19	31	69	0

Table 36: Results of PVX resistance tests in the Desiree x
Cara cross

	number	percentage		
	of plants	established		
PI	20	65%		
C	20	80%		
PIxPI	100	52%		
CxC	100	85%		
F ₁ PIxC	50	86%		
M ₁ PIxC-5	38	32%		
M ₁ PIxC-15	16	50%		
			new families	
F ₂ PIxC	282	60%	284	5%
M ₂ PIxC-5	38	50%	64	2%
M ₂ PIxC-15	46	30%	98	21%

Table 37: Establishment 18 days after planting in the
Pentland Ivory x Cara cross

	number of plants	percentage established		
D	20	95%		
C	20	80%		
DxD	100	75%		
CxC	100	85%		
F ₁ DxC	50	76%		
M ₁ DxC-5	50	46%		
M ₁ DxC-15	14	57%		
			new families	
F ₂ DxC	178	81%	150	1%
M ₂ DxC-5	50	70%	50	1%
M ₂ DxC-10	-	-	50	2%

Table 38: Establishment 18 days after planting in the
Desiree x Cara cross

	number of plants	percentage established		
D	20	95%		
PI	20	65%		
DxD	100	75%		
PIxPI	100	52%		
F ₁ DxPI	46	93%		
M ₁ DxPI-5	50	42%		
			new families	
F ₂ DxPI	350	57%	200	4%
M ₂ DxPI-5	24	13%	100	1%

Table 39: Establishment 18 days after planting in the
Desiree x Pentland Ivory cross

When the M_1 s were compared to their respective M_2 s, no significant differences were found (5 Krad $\chi^2 = 1.84$, 15 Krad $\chi^2 = 1.98$). At first sight this may appear to support the notion that radiation induced damage persists. But when the controls were examined, the F_2 was found to be significantly later emerging than the F_1 ($\chi^2 = 12.79^{***}$), perhaps reflecting the effects of inbreeding.

Such a difference would also have been expected between the irradiated generations. The fact that the M_2 was not significantly lower scoring than M_1 may indicate that the effects of inbreeding had been balanced by the elimination of radiation damage.

The results for the second cross, Desiree x Cara are presented in Table 38. Again F_1 plants tended to emerge before those of the M_1 , a characteristic which was significant in the case of the 5 Krad treatment ($\chi^2 = 25.49^{***}$) but not for the 10 Krad group where the sample size was small ($\chi^2 = 2.73$). Likewise, the F_2 produced top growth before the M_2 , although the difference was not as great and only just significant at the 5% level ($\chi^2 = 3.84^*$). Again there was not a significant difference between the F_2 and the maternal self ($\chi^2 = 1.35$).

Unlike the first cross, the F_1 and F_2 scored similarly for establishment ($\chi^2 = 0.52$), whereas the M_2 emerged earlier than the M_1 ($\chi^2 = 5.91^*$). Again this may indicate that radiation damage had been preferentially eliminated.

In the third cross (Desiree x Pentland Ivory, Table 39), first generation plants tended to emerge before their second generation derivatives (F_1 & F_2 $\chi^2 = 26.48^{***}$; M_1 & M_2 $\chi^2 = 17.49^{***}$). And the controls emerged before their irradiated counterparts (F_1 & M_1 $\chi^2 = 217.41^{***}$; F_2 & M_2 $\chi^2 = 18.01^{***}$). This time there was a difference between the F_2 and the maternal self, the latter emerging significantly earlier ($\chi^2 = 10.13^{***}$). As the M_2 was slower to become established than the F_2 , the shift was actually away from the maternal self.

	\bar{x}		SEM
PI	32.60	\pm	1.44
C	20.15	\pm	1.38
PIxPI	23.05	\pm	1.28
CxC	20.82	\pm	0.66
F ₁ PIxC	27.08	\pm	1.09
M ₁ PIxC-5	21.71	\pm	1.36
M ₁ PIxC-15	24.56	\pm	2.76
F ₂ PIxC	17.79	\pm	0.40
M ₂ PIxC-5	17.91	\pm	1.27
M ₂ PIxC-15	15.11	\pm	0.96

Table 40: Plant heights (cm) 32 days after planting in
the Pentland Ivory x Cara cross

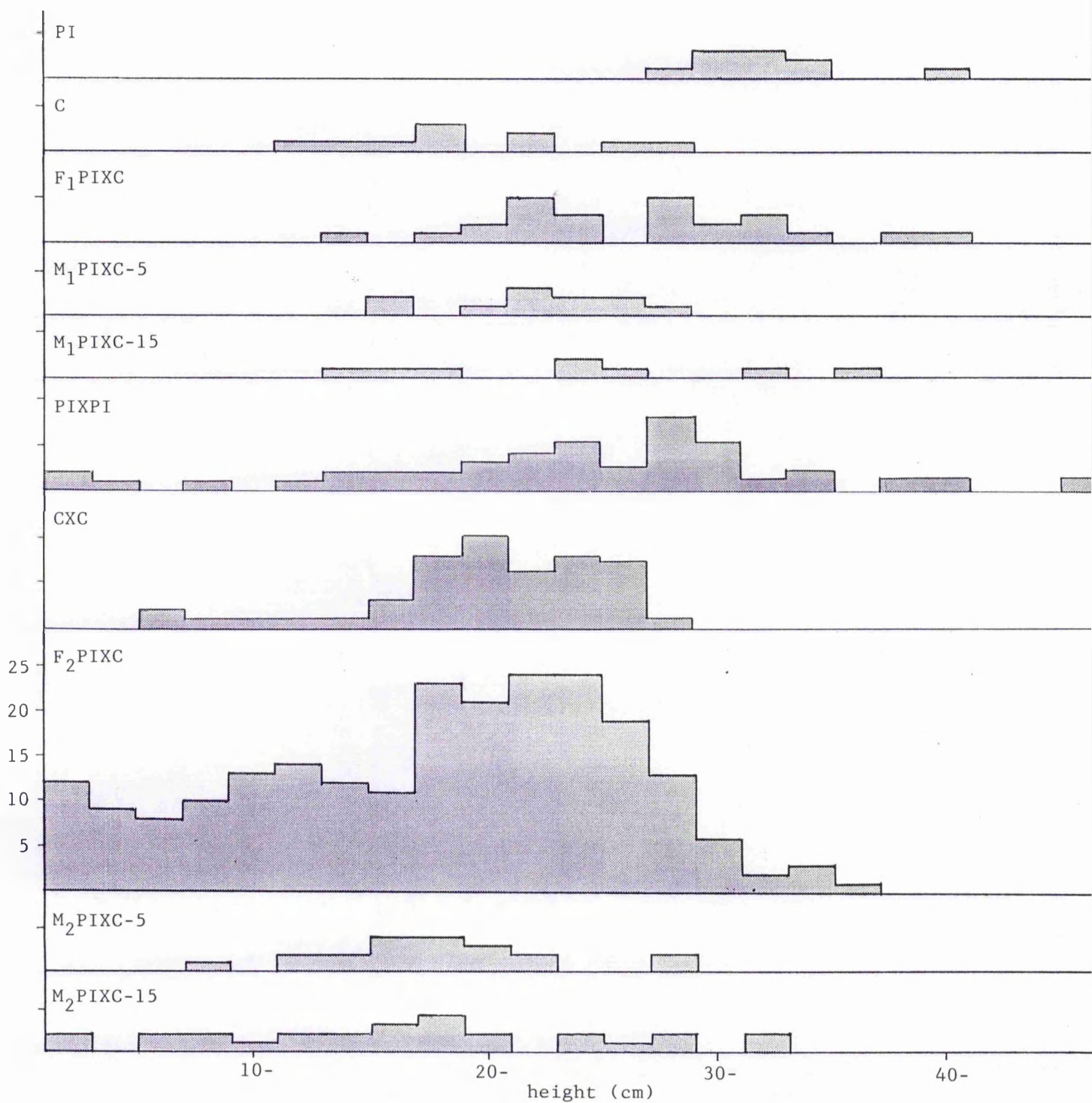


Figure 44: Distribution of clone means for height in the PIXC cross

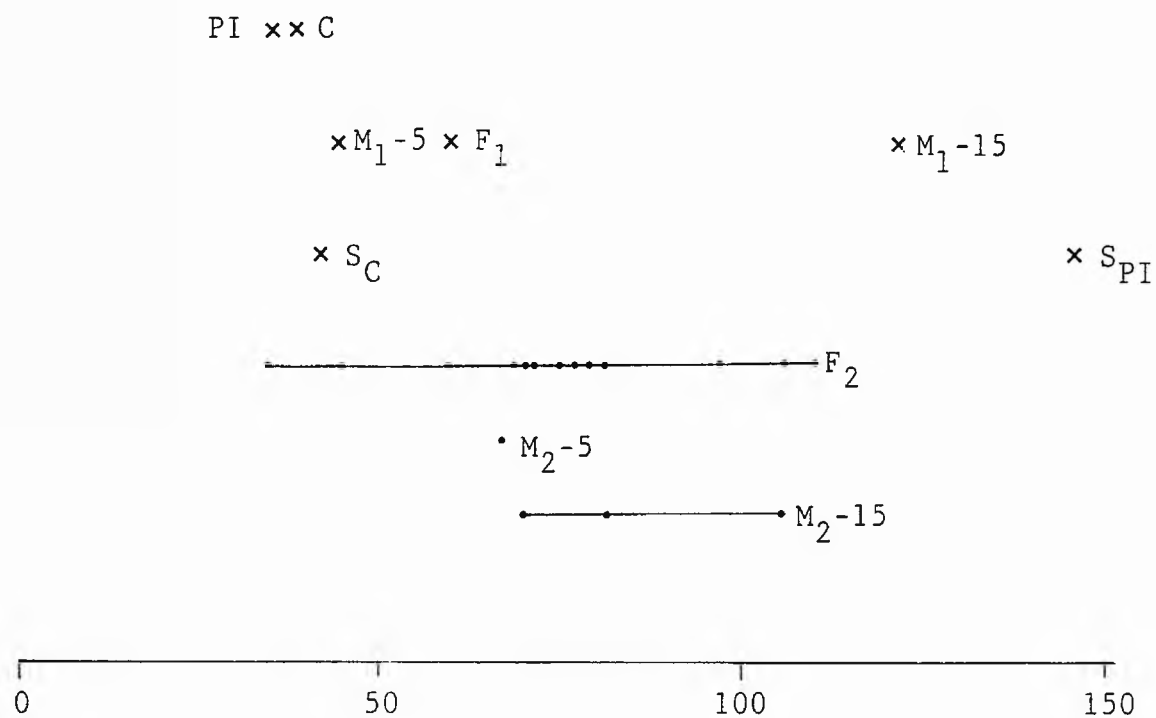


Figure 45: Variance distribution for height in the PIXC cross

- height

i) Pentland Ivory x Cara

The height data for this cross are summarised in Table 40 as treatment means with their standard errors (see Appendix C1 for more detailed results). Histograms of clone means are displayed in Figure 44, while the distribution of variances is plotted in Figure 45.

Inbred generations tended to be shorter than the outcrossed ones, and plants derived from irradiated crosses were generally shorter than the controls. Because height at 32 days was, to some extent, also a measure of establishment, dormancy may again have confused interpretation of the results. For this reason, the means of new and old second generation families have been separated in the following table.

	old	new
PI x PI	23.05 \pm 1.28	
F ₂	20.98 \pm 0.52	13.48 \pm 0.62
M ₂ -5	17.91 \pm 1.27	
M ₂ -15	13.93 \pm 1.59	15.99 \pm 1.17

The newer F₂ families were indeed shorter than the older ones. But, surprisingly, this was not true for the M₂ at 15 Krads. To avoid confusion, the discussion that follows is confined to the older families.

PI x PI, the maternal self, was marginally taller than the F₂. So, had genomic selection been dominant in determining M₂ phenotype, this generation would also have been slightly taller than the control. In fact, the reverse was the case, plants becoming shorter as dose increased.

In addition, if mutational damage had been eliminated, it might be apparent in the ratio of the scores of first generation plants to those of second generation families derived from them (it would be lower for the treated groups). In fact, the ratio was slightly higher in the irradiated crosses:-

O Krads	5 Krads	15 Krads
1.37	1.57	1.78

The distributions plotted in Figure 44 show that the range of heights were similar for the F_1 and the M_1 , as well as for the F_2 and the M_2 . The inbred generations, including the parental selfs, produced lower scoring clones (although in the F_2 this was partly due to dormancy in the newer families). The maternal self included some of the shortest and the tallest clones, a characteristic not mirrored by the M_2 s. The distribution of variances (figure 45) shows the range for the M_2 was contained within that of the F_2 and it did not approach the high variance of the maternal self.

ii) Desiree x Cara

The results for height in this cross are summarised in Table 41, and in Figures 46 and 47. The means of the new and old second generation families and the maternal self are given below.

	old	new
DXD	22.23 \pm 0.72	
F_2	20.78 \pm 0.54	9.41 \pm 0.99
M_2 -5	24.73 \pm 1.01	
M_2 -10		11.12 \pm 1.46

While the M_1 -5Krads was shorter than the F_1 , in the second generation the position had reversed. When it came to the 10 Krad treatment, means were statistically indistinguishable from the relevant controls in both generations. The maternal self was marginally taller than the F_2 , and the M_2 -5Krads was taller again. In fact, it was statistically indistinguishable from Desiree, the maternal parent (Table 41).

	\bar{x}		SEM
D	25.20	\pm	1.12
C	20.15	\pm	1.38
DxD	22.23	\pm	0.72
CxC	20.82	\pm	0.66
F ₁ DxC	22.44	\pm	0.86
M ₁ DxC-5	18.04	\pm	1.74
M ₁ DxC-10	20.14	\pm	1.63
F ₂ DxC	17.22	\pm	0.48
M ₂ DxC-5	24.73	\pm	1.01
M ₂ DxC-10	11.12	\pm	1.46

Table 41: Plant heights (cm) 32 days after planting in
the Desiree x Cara cross

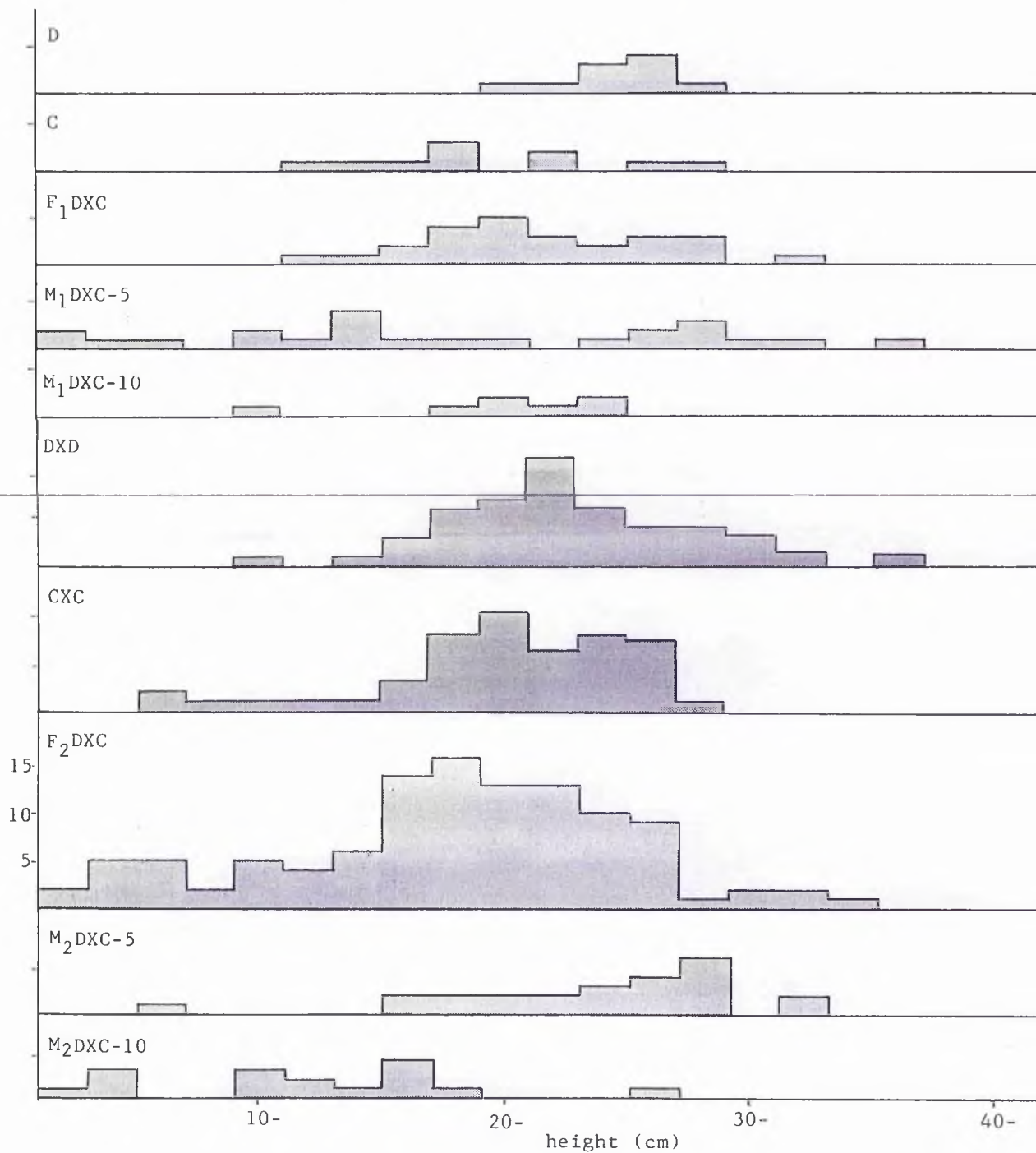


Figure 46 : Distribution of clone means for height in the DXC cross

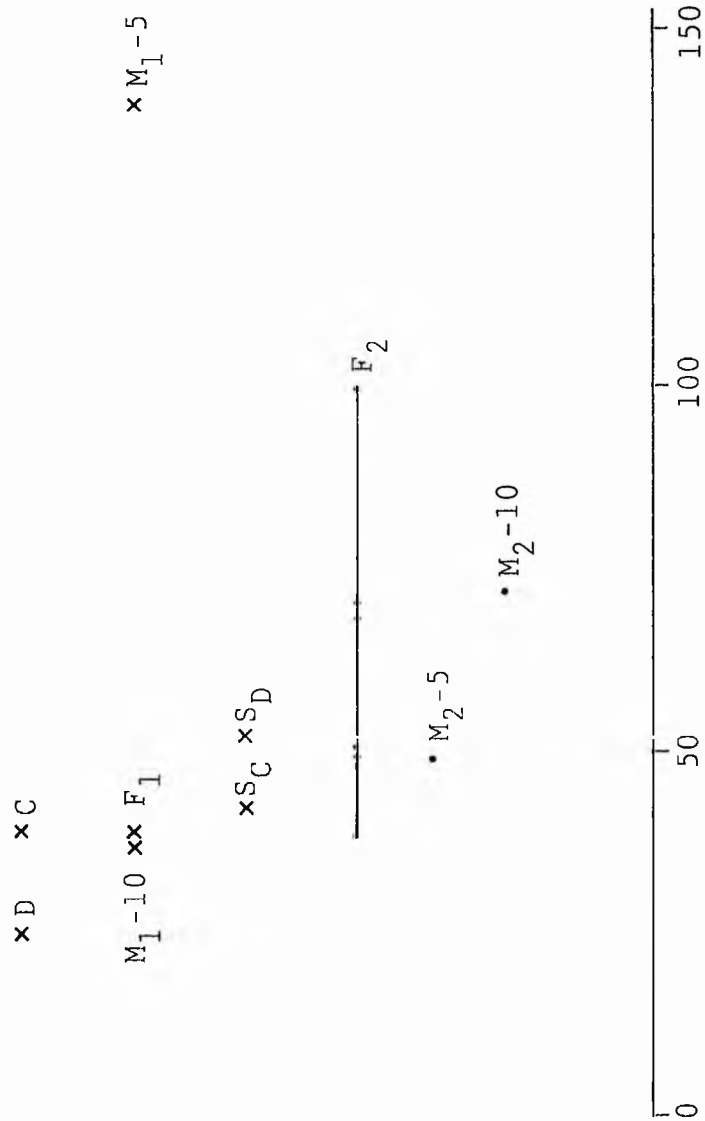


Figure 47: Variance distribution for height in the DXC cross

The ratios of the first generation plant heights to their second generation derivatives (1.11 for the control, 1.01 for the 5 Krad treatment) suggest some elimination of radiation induced damage may have taken place during the formation of the M_2 . While this phenomenon seems to be evident in Figure 46 (the M_1 -5 having had some lower scoring clones than the respective M_2), only one family is represented in the M_2 histogram and it was derived from a relatively high scoring M_1 plant (25cm). Again, the low scores of some of the second generation clones were due to dormancy in the newer families.

The variability displayed by the parents, that within first generation treatment groups, and that within second generation families is shown in Figure 47. Apart from the highly variable M_1 at the 5 Krad dose, variances were similar for the parents, parental selfs and first generation hybrids. As scores were limited to two M_2 families for this character (one family having failed to emerge by 32 days), and as both fell within the F_2 variability range, little can be drawn from the second generation results.

iii) Desiree x Pentland Ivory

The height data from the third cross are summarised in Table 42, and in Figures 48 and 49. Both parents were taller than their selfs, as were the F_1 and the M_1 . The control groups in both generations were taller than their irradiated counterparts. Means and standard errors of the means for second generation families and the maternal self are given below:

	old	new
D X D	22.23 \pm 0.72	
F_2	22.65 \pm 0.42	12.10 \pm 0.89
M_2 -5	14.67 \pm 2.27	12.14 \pm 1.60

	\bar{x}		SEM
D	25.20	\pm	1.12
PI	32.60	\pm	1.44
DxD	22.23	\pm	0.72
PIxPI	23.05	\pm	1.28
F ₁ DxPI	25.98	\pm	0.91
M ₁ DxPI-5	21.44	\pm	1.31
F ₂ DxPI	20.11	\pm	0.37
M ₂ DxPI-5	13.28	\pm	1.35

Table 42: Plant heights (cm) 32 days after planting in
the Desiree x Pentland Ivory cross

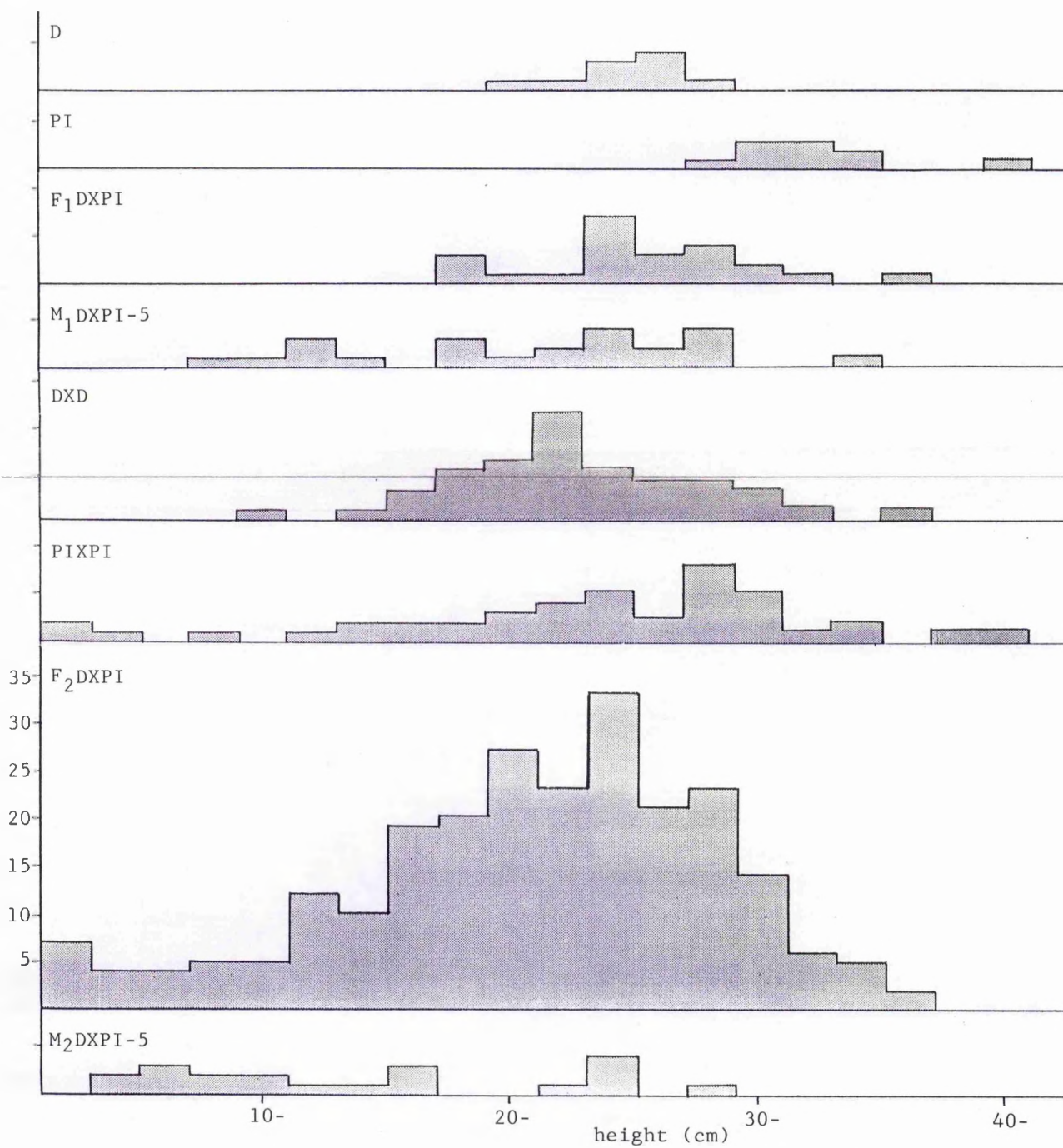


Figure 48: Distribution of clone means for height in the DXPI cross

1. The first part of the document is a list of the names of the persons who have been appointed to the various positions of the Board of Directors of the Corporation.

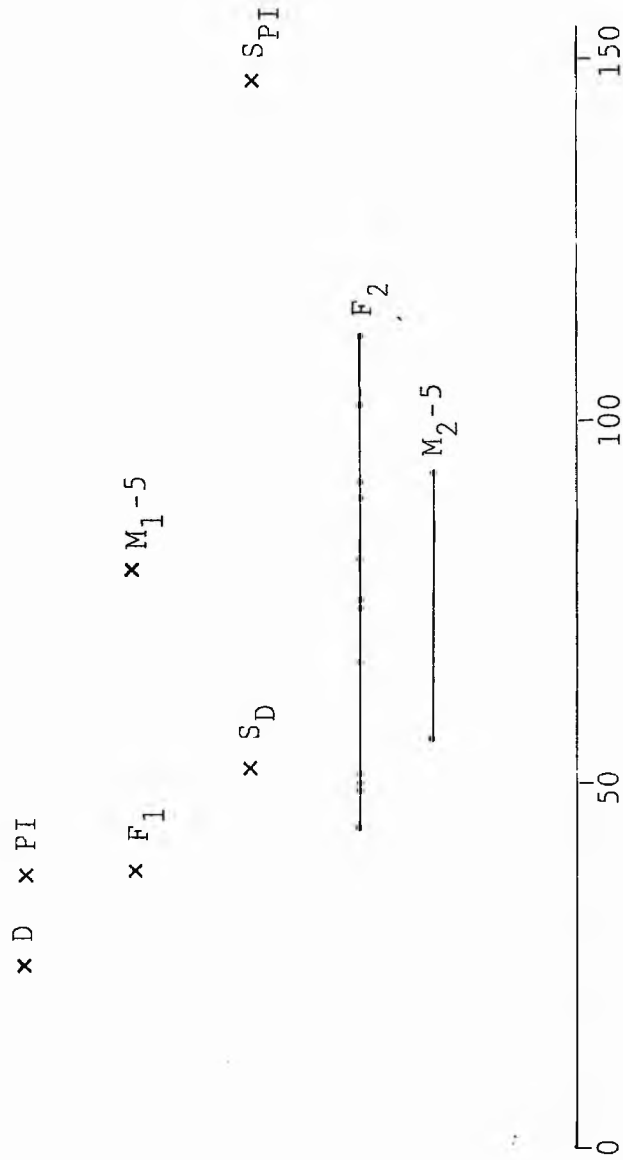


Figure 49: Variance distribution for height in the DXPI cross

Again, the following discussion concentrates on old families. The F_2 was statistically indistinguishable from the maternal self, but the M_2 was considerably shorter. When the ratios of 1st to 2nd generation heights were examined (0 Krads - 1.12, 5 Krads - 1.91), they too suggested a large amount of radiation damage may have been present in the M_2 .

Once more, the small number of families and the problem with dormancy complicates interpretation of the distributions. Even so, both the clone means (Figure 48) and the variances of the M_2 (Figure 49) were within the range expressed by the F_2 .

- tuber number

i) Pentland Ivory x Cara

Both parents produced comparable numbers of tubers, and neither was distinguishable from its self for this character (Table 43). Because the parental selfs would be expected to be suffering the ill effects of inbreeding, the absence of significant differences between these generations highlights the fact that tuber number on its own is not necessarily a good measure of fitness. Nevertheless, the difference between the F_2 and the maternal self for tuber number meant the character could have been used to distinguish maternal trends in the M_2 had they occurred.

In the event, the M_2 produced fewer tubers than the F_2 even though the maternal self had produced more. And likewise, the M_1 produced fewer tubers than F_1 .

When it came to the distribution of clone means (Figure 50), the most noticeable feature was the downward skew of the F_2 and M_2 histograms. This did not appear to be a characteristic of the other inbred generations, the parental selfs. As far as variability was concerned (Figure 51), while the M_1 s were more and less variable than the F_1 , M_2 families were located at the lower end of the F_2 distribution.

	\bar{x}		SEM
PI	4.81	\pm	0.29
C	4.55	\pm	0.51
PIxPI	4.38	\pm	0.28
CxC	4.91	\pm	0.30
F ₁ PIxC	5.26	\pm	0.36
M ₁ PIxC-5	4.47	\pm	0.69
M ₁ PIxC-15	3.50	\pm	0.54
F ₂ PIxC	3.51	\pm	0.08
M ₂ PIxC-5	2.50	\pm	0.16
M ₂ PIxC-15	3.15	\pm	0.17

Table 43: Tuber numbers in the Pentland Ivory
x Cara cross

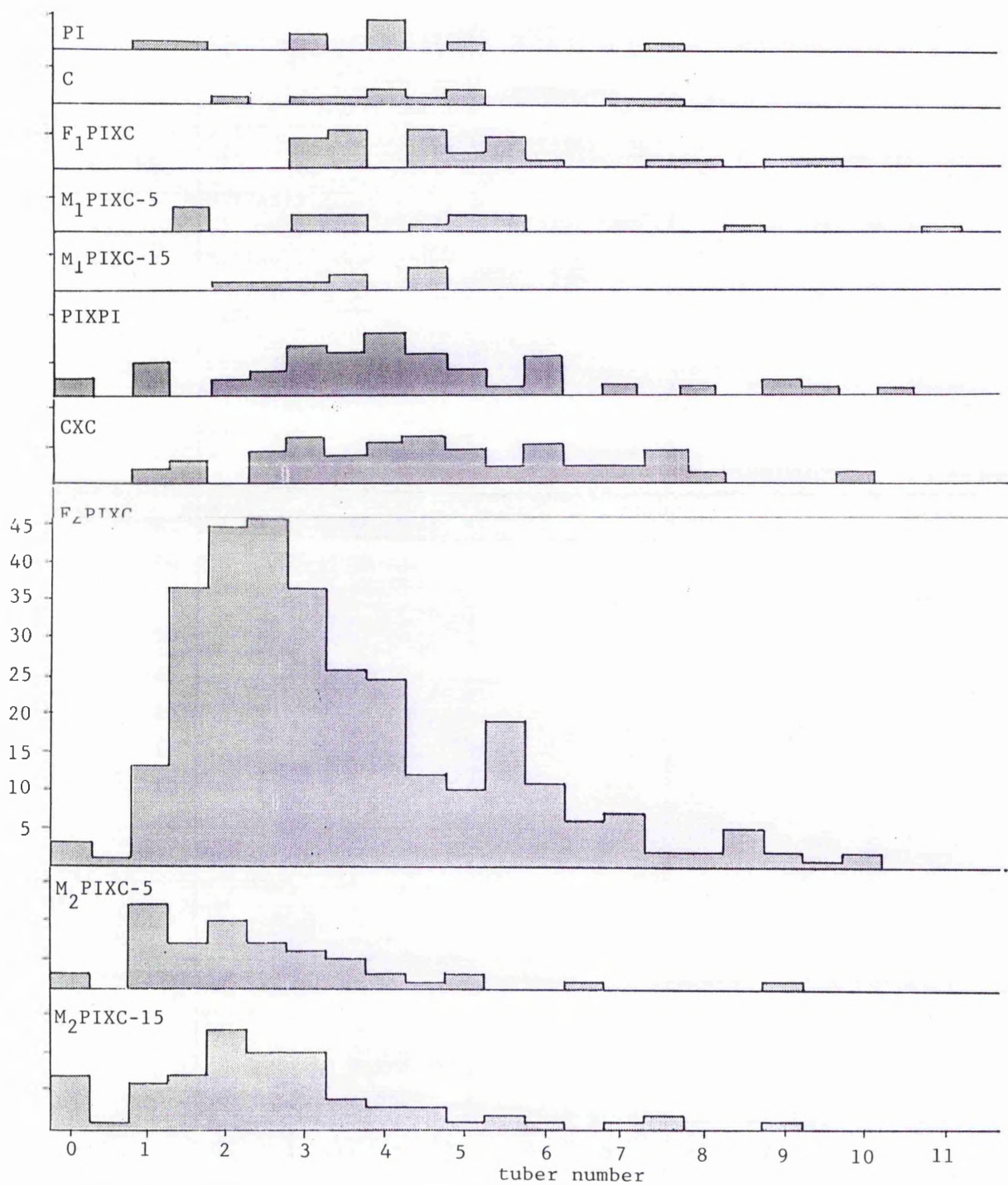


Figure 50: Distribution of clone means for tuber number in the PIXC cross

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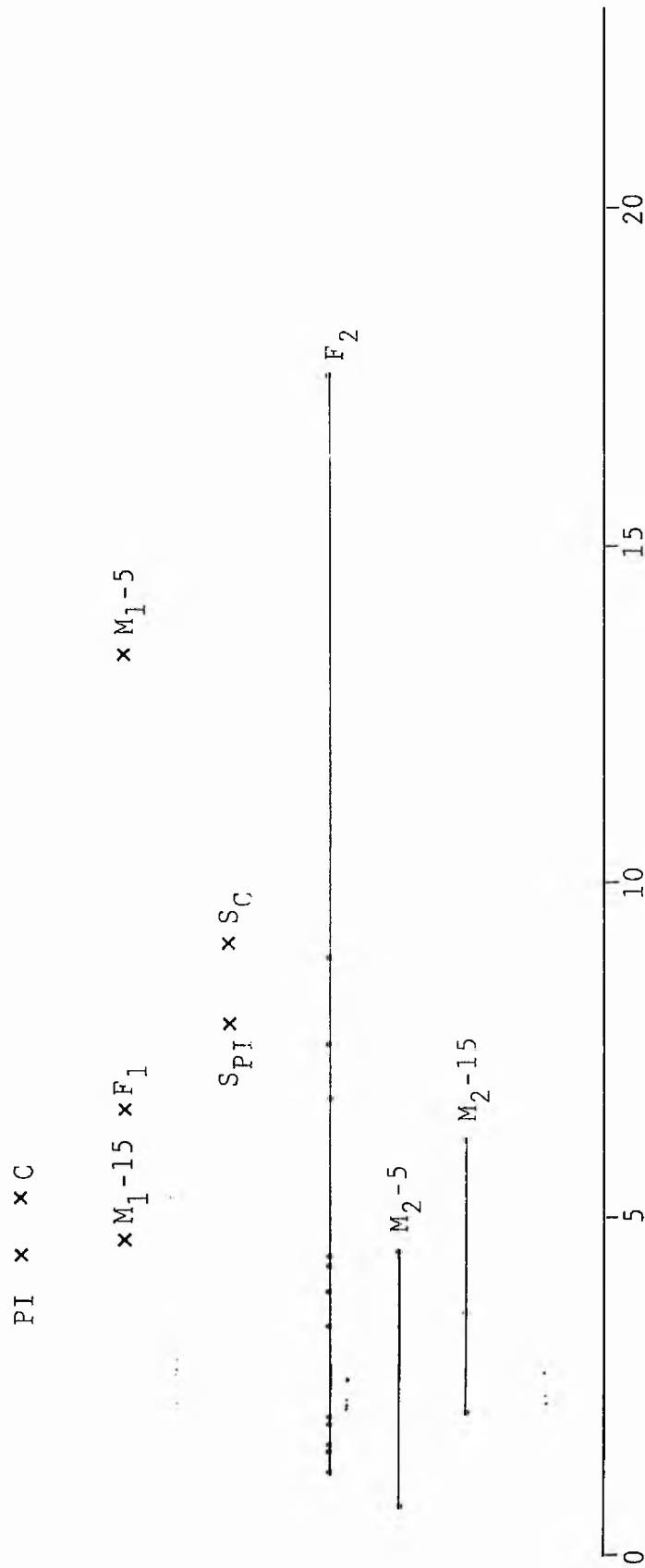


Figure 51: Variance distribution for tuber number in the PIXC cross

ii) Desiree x Cara

These results followed the pattern set in the Pentland Ivory X Cara cross. That is, the parents were statistically indistinguishable from each other and their selfs. The M_1 produced fewer tubers than the F_1 , although the deficit was only significant in the case of the 10 Krad treatment. Similarly, the M_2 produced fewer tubers than the F_2 which produced fewer tubers than the maternal self (Table 44).

Just as in the first cross, an excess of lower scoring clones was evident in the M_2 and F_2 generations (Figure 52). And once again, the most noticeable feature of the variance distribution (Figure 53) was the location of the M_2 families at the lower end of the variance spectrum.

iii) Desiree x Pentland Ivory

The third cross also gave rise to a similar pattern of results for tuber number (Table 45, Figures 54 and 55). The F_1 tended to produce more tubers than the M_1 , although not significantly so. But in the second generation the excess of the control over the treated group was statistically significant. Again, the movement in the M_2 was away from the maternal self. The distributions (Figures 44 and 45) were also in keeping with earlier findings for this character. That is, there was an excess of low scoring clones in the F_2 and in the M_2 . And M_2 families were less variable than many of their F_2 counterparts.

- tuber weight

i) Pentland Ivory x Cara

The data for this cross are presented in Table 46 and in Figures 56 and 57. Unlike tuber number, the weight of tubers produced was much reduced in the parental selfs, falling to almost 50% of the parental values. By contrast, tuber weight was maintained in the F_1 , although it fell sharply in the M_1 . In the inbred F_2 and M_2 scores were once again much lower, falling further as dose increased.

	\bar{x}		SEM
D	5.00	\pm	0.61
C	4.55	\pm	0.51
DxD	4.51	\pm	0.26
CxC	4.91	\pm	0.30
F ₁ DxC	4.38	\pm	0.35
M ₁ DxC-5	4.18	\pm	0.33
M ₁ DxC-10	3.93	\pm	0.82
F ₂ DxC	3.55	\pm	0.11
M ₂ DxC-5	2.57	\pm	0.14
M ₂ DxC-10	3.19	\pm	0.28

Table 44: Tuber numbers in the Desiree x Cara cross

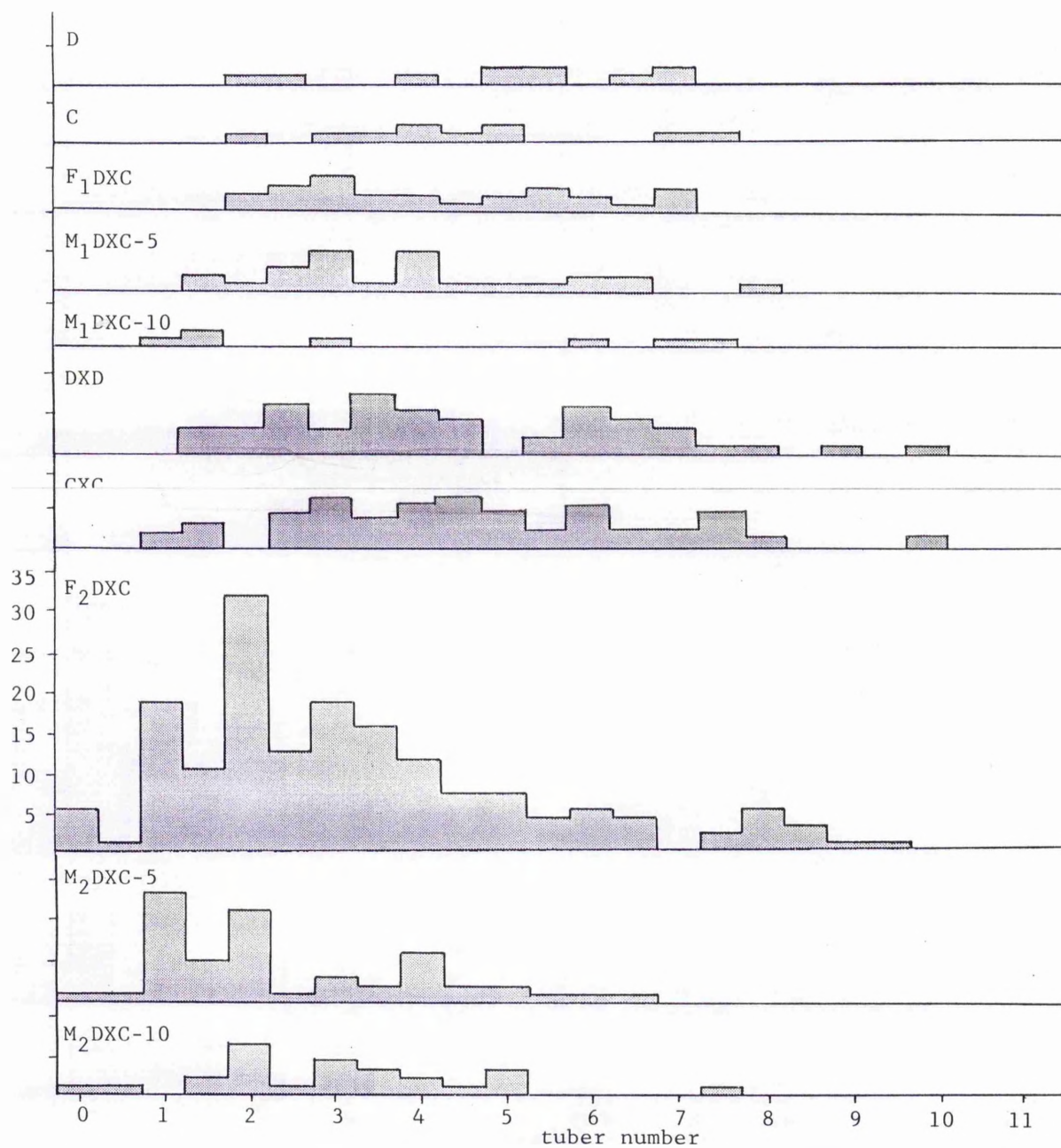


Figure 52: Distribution of clone means for tuber number in the DXC cr

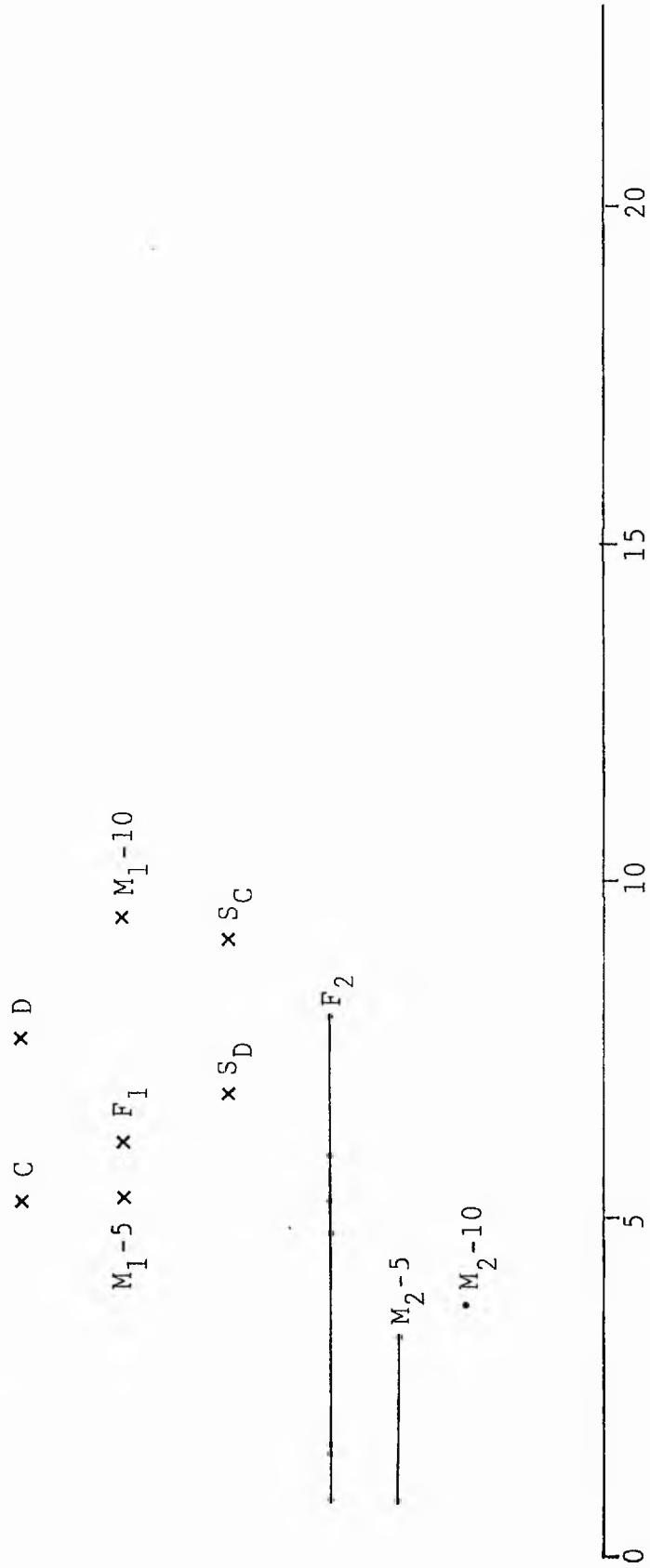


Figure 53: Variance distribution for tuber number in the DXC cross

	\bar{x}		SEM
D	5.00	\pm	0.61
PI	4.81	\pm	0.51
DxD	4.51	\pm	0.26
PIxPI	4.38	\pm	0.28
F ₁ DxPI	5.39	\pm	0.41
M ₁ DxPI-5	4.77	\pm	0.48
F ₂ DxPI	3.58	\pm	0.10
M ₂ DxPI-5	2.36	\pm	0.13

Table 45: Tuber numbers in the Desiree
x Pentland Ivory cross

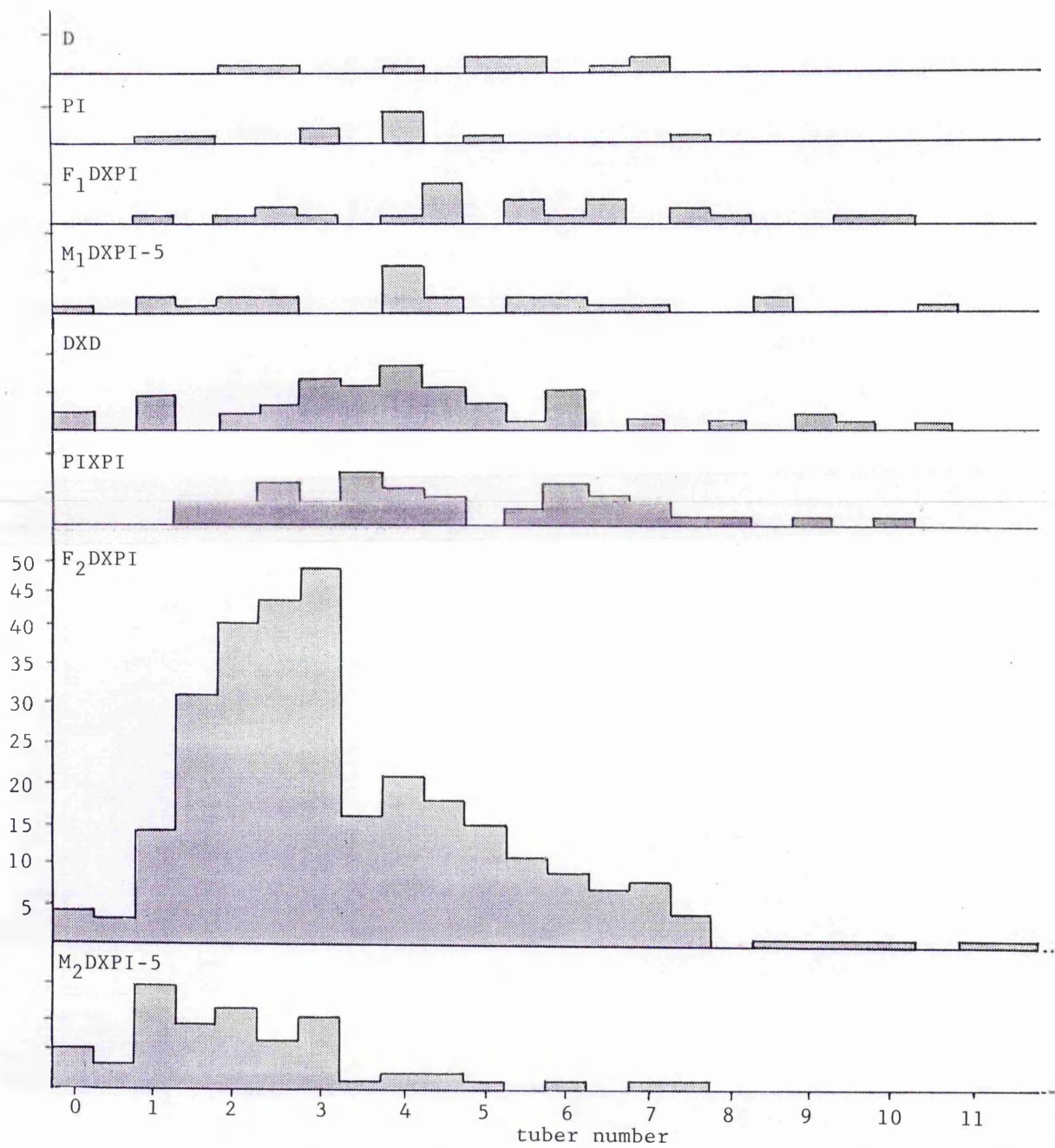


Figure 54: Distribution of clone means for tuber number in the DXPI

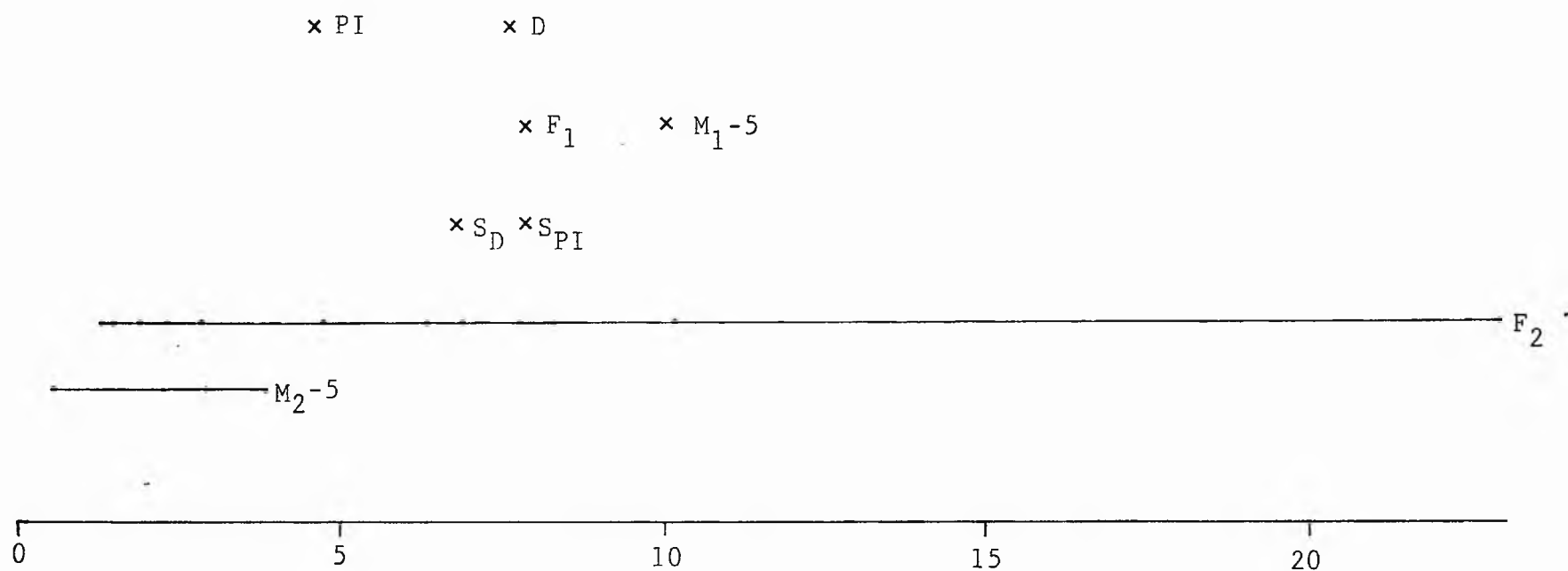


Figure 55: Variance distribution for tuber number in the DXPI cross

	\bar{x}		SEM
PI	41.61	\pm	4.13
C	41.57	\pm	3.35
PIxPI	22.37	\pm	1.34
CxC	23.18	\pm	1.87
F ₁ PIxC	42.41	\pm	3.02
M ₁ PIxC-5	27.53	\pm	3.41
M ₁ PIxC-15	23.89	\pm	2.81
F ₂ PIxC	17.31	\pm	0.39
M ₂ PIxC-5	10.95	\pm	0.73
M ₂ PIxC-15	9.45	\pm	0.71

Table 46: Tuber weights (g) in the Pentland Ivory x
Cara cross

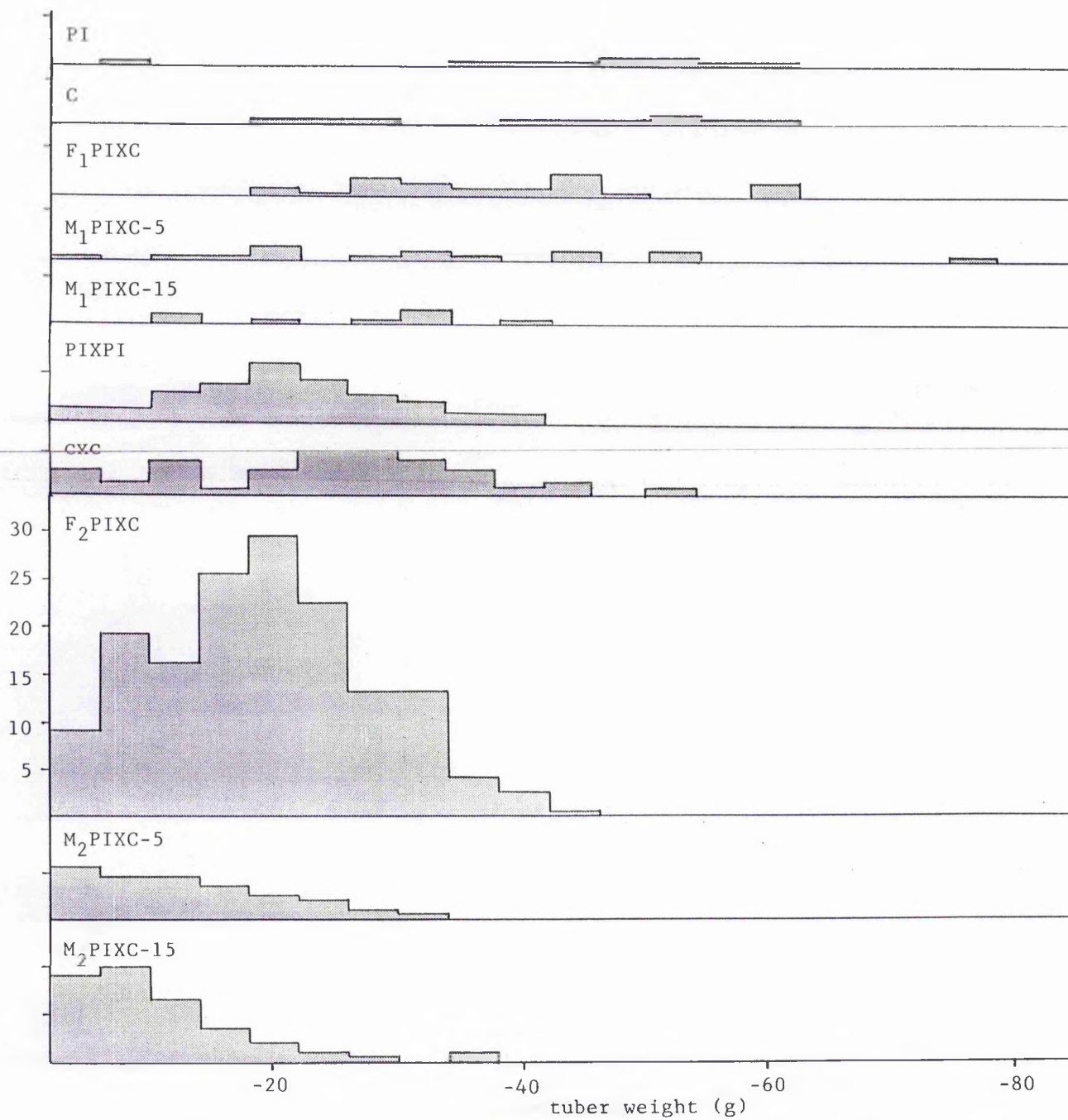


Figure 56: Distribution of clone means for tuber weight in the PIXC cross

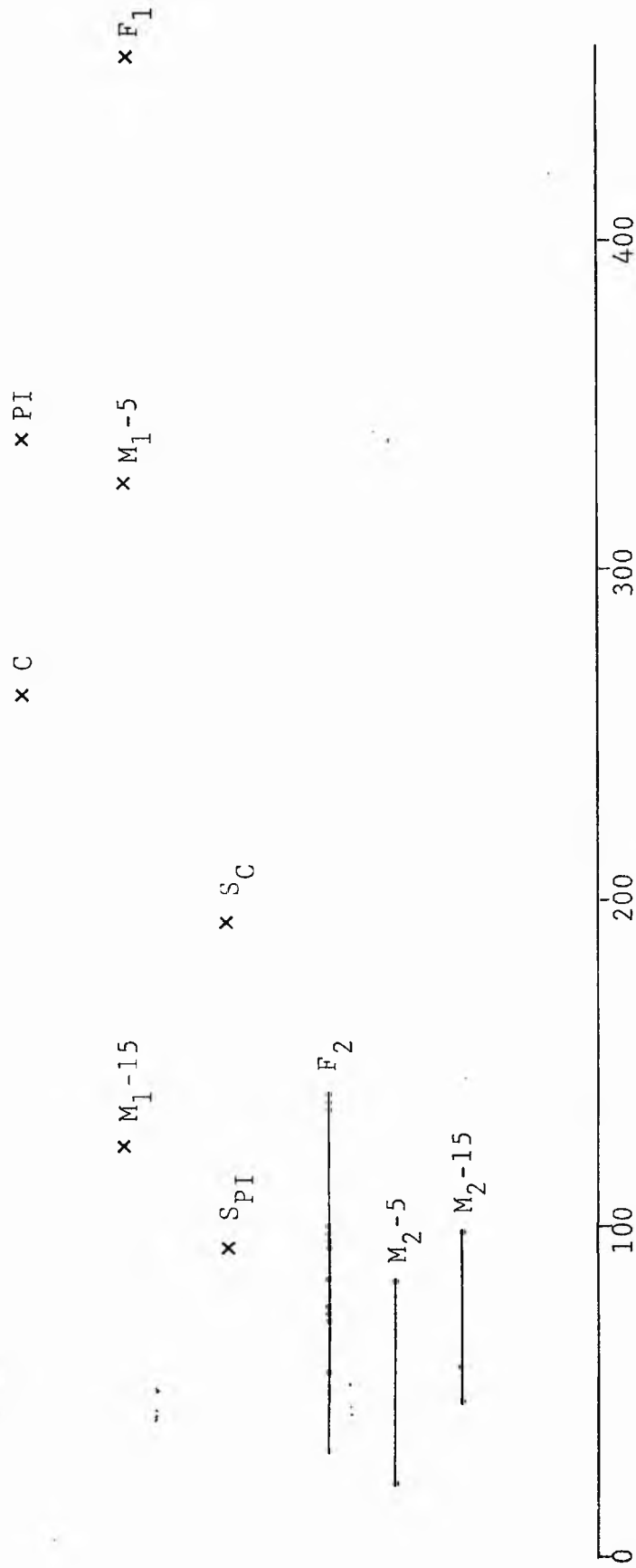


Figure 57: Variance distribution for tuber weight in the PIXC cross

The tubers produced by the maternal self, PI x PI, weighed on average marginally more than those of the F_2 . So the reduction observed in the M_2 was consistent with radiation damage persisting rather than it having been eliminated. In the case of the 5 Krad treatment, this explanation is also supported by the 1st/2nd generation ratios:

O Krad	5 Krad	15 Krad
2.78	4.32	2.30

As the control ratio is a guide to the effects of inbreeding, and as the 15 Krad ratio is lower than that of the control, some radiation damage may have been eliminated during the formation of the 15 Krad M_2 . But this was not enough to cause a shift from the F_2 to the maternal self.

An examination of the histograms of mean weights (Figure 56) shows the distributions of all the inbred generations had a downward skew. This was more marked in the M_2 than the F_2 or the parental selfs, presumably because the effects of inbreeding were compounded by those of the radiation treatment. Perhaps surprisingly, the highest scoring clones were irradiated hybrids (M_1 -5 Krads).

Although every effort was made to select tubers of similar size and condition, there were bound to be differences that may have had a bearing on subsequent growth. This environmental factor was probably responsible for the relatively high variability of the parents and the F_1 . Indeed, when the mean score for each F_1 clone was plotted (Figure 56), the spread was actually less than that of the M_1 -5 Krads which had a lower variance.

ii) Desiree x Cara

Means with their standard errors are given in Table 47, while histograms of tuber weight and variances are displayed in Figures 58 and 59 respectively. As far as the parents, the parental selfs and the first generation hybrids were concerned, the results for this cross followed the pattern of those in the first cross. But they differed slightly in the second generation. Again the F_2 was lower scoring than the maternal self. But although the M_2 was lower scoring than the F_2 , this phenomenon was only statistically distinguishable at the 5 Krad dose.

The ratio of tuber weights produced by first generation plants to those of second generation families they gave rise to are given below:

0 Krads	5 Krads	10 Krads
2.31	2.11	1.79

The lower ratios in the irradiated groups suggest some elimination of radiation damage took place during the formation of the M_2 . However, it was of insufficient magnitude to cause a shift in this generation towards the maternal self away from the F_2 .

The distributions of clone means (Figure 58) shows that low scoring clones occurred in all but the parental groups and the F_1 . Despite the M_1 being generally lower scoring than the F_1 , one clone achieved the equal highest score. Once again, variability within M_2 families was low, the range being at the bottom end of that of the F_2 (Figure 59).

iii) Desiree x Pentland Ivory

Just as in the other two crosses, inbred generations tended to be lower scoring than the parents and the outcrossed generations (Table 48). The difference between plants derived from irradiated crosses and the controls was particularly marked, both the M_1 and the M_2 producing roughly half the weight of tubers per plant that the F_1 and F_2 produced respectively.

	\bar{x}		SEM
D	43.19	\pm	4.09
C	41.57	\pm	3.35
DxD	25.72	\pm	1.36
CxC	23.18	\pm	1.87
F ₁ DxC	35.42	\pm	2.08
M ₁ DxC-5	27.32	\pm	2.18
M ₁ DxC-10	17.82	\pm	2.69
F ₂ DxC	18.57	\pm	0.60
M ₂ DxC-5	14.72	\pm	0.82
M ₂ DxC-10	17.66	\pm	1.34

Table 47: Tuber weights (g) in the Desiree x Cara cross

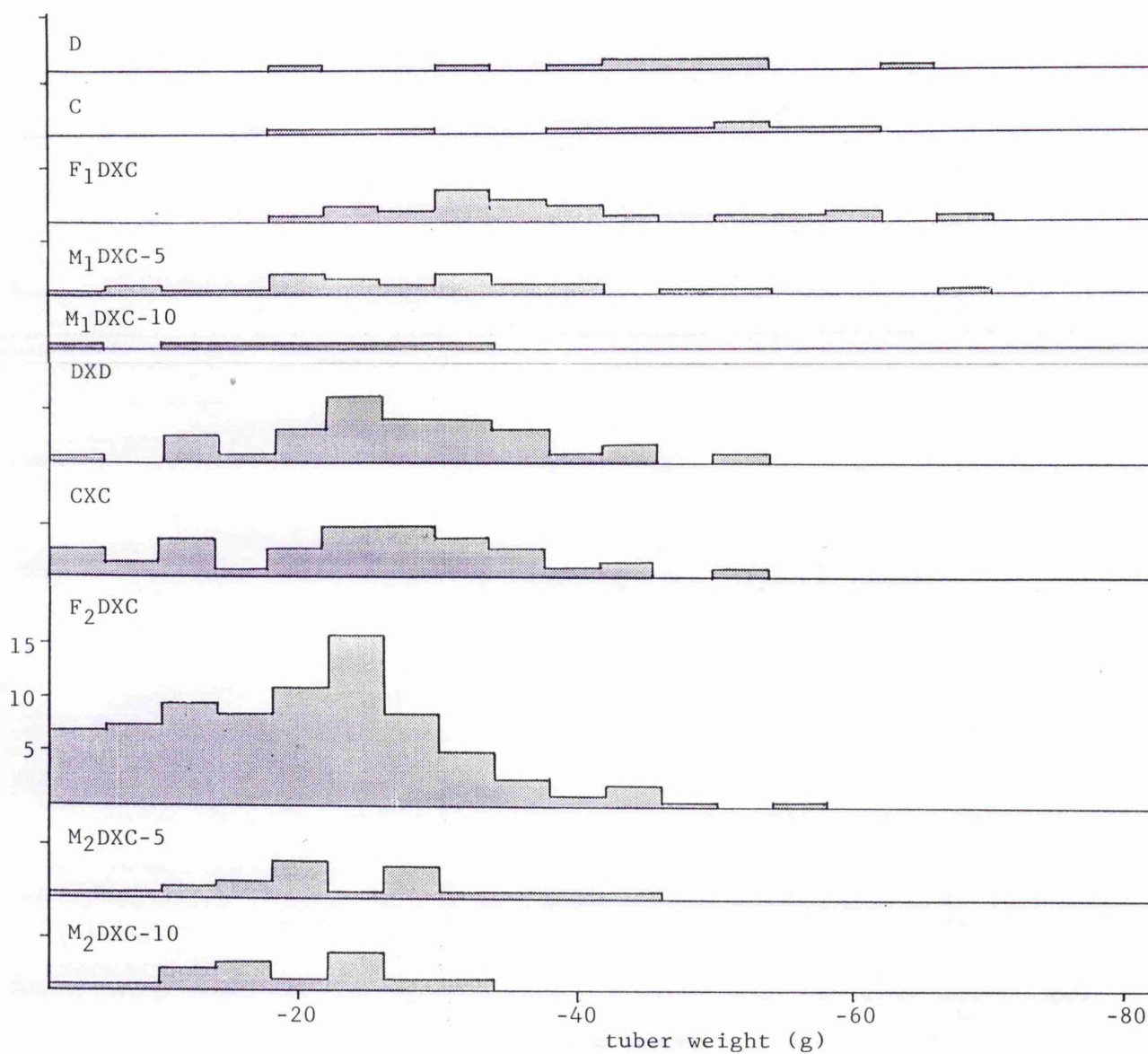


Figure 58: Distribution of clone means for tuber weight in the DXC

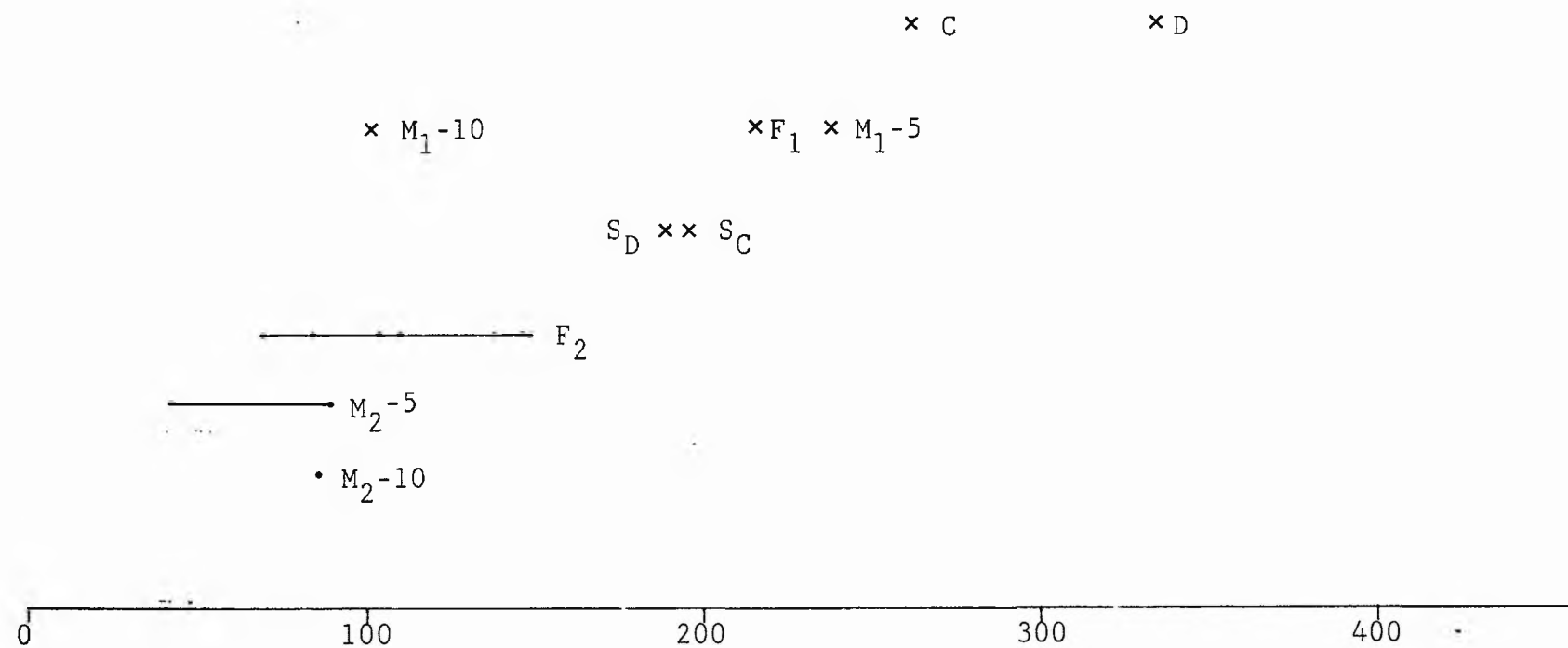


Figure 59 : Variance distribution for tuber weight in the DXC cross

	\bar{x}		SEM
D	43.19	<u>+</u>	4.09
PI	41.61	<u>+</u>	4.13
DxD	25.72	<u>+</u>	1.36
PIxPI	22.37	<u>+</u>	1.34
F ₁ DxPI	40.72	<u>+</u>	2.77
M ₁ DxPI-5	20.01	<u>+</u>	1.85
F ₂ DxPI	18.66	<u>+</u>	0.42
M ₂ DxPI-5	8.31	<u>+</u>	0.65

Table 48: Tuber weights (g) in the Desiree x
Pentland Ivory cross

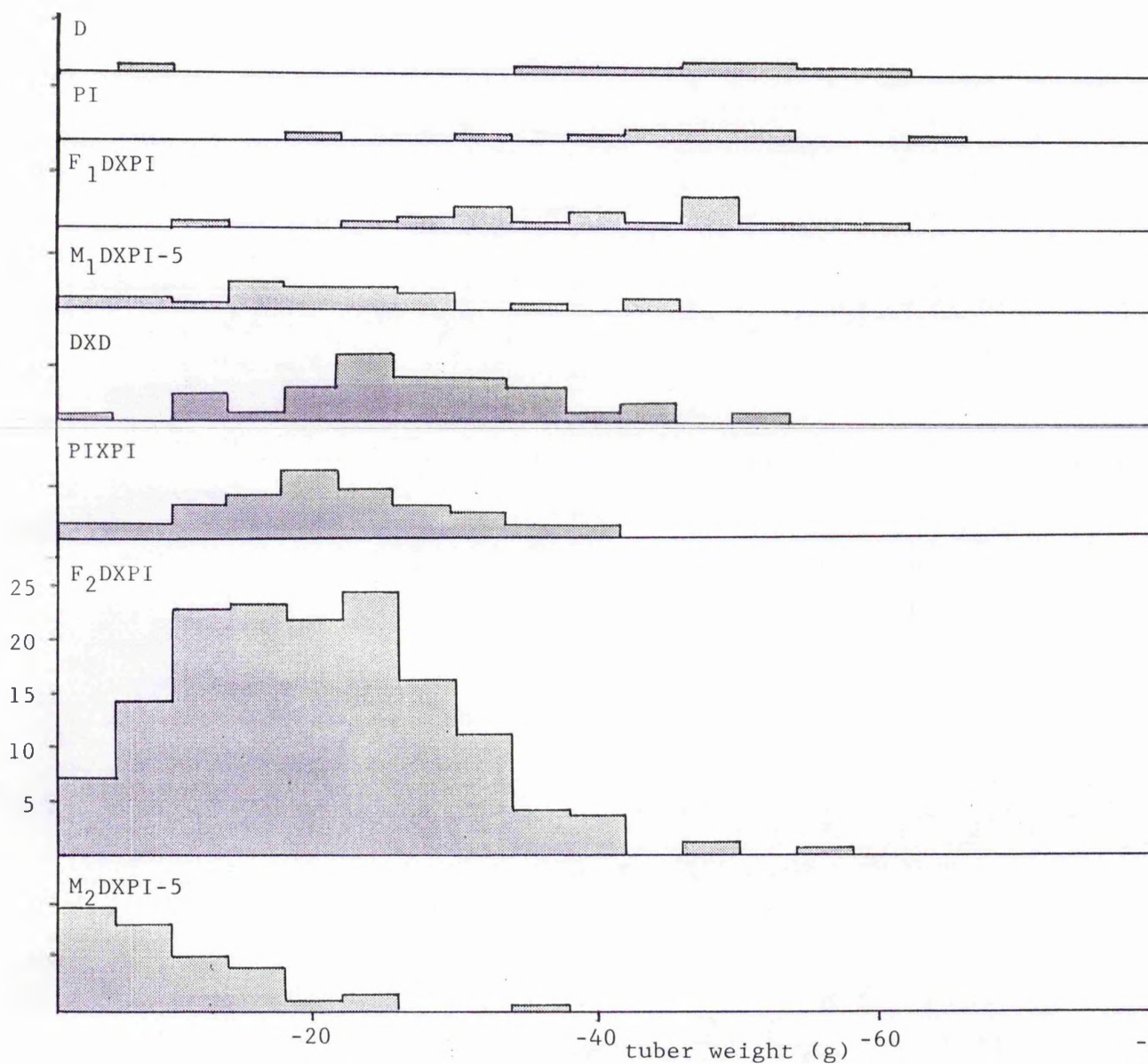


Figure 60 : Distribution of clone means for tuber weight in the DXPI

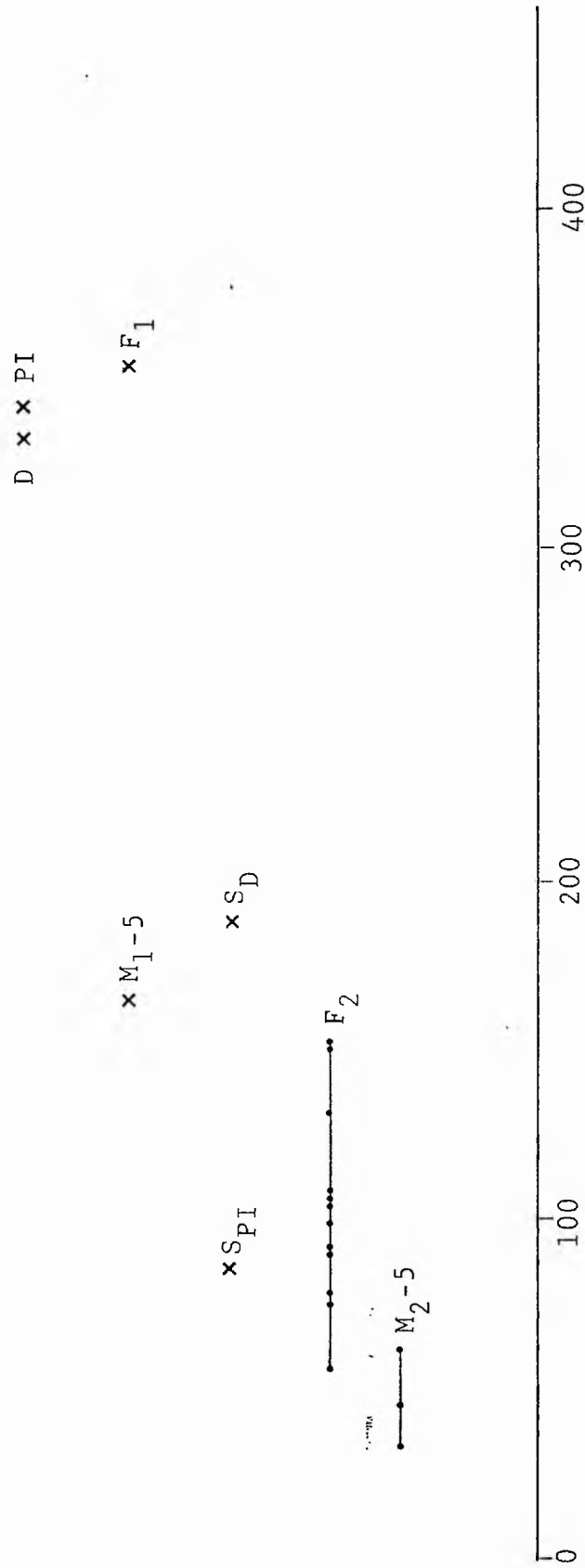


Figure 61: Variance distribution for tuber weight in the DXPI cross

As the maternal self was higher scoring than the F_2 , the relatively low score of the M_2 was again a shift away from the maternal. The first to second generation ratios (0 Krads = 2.37, 5 Krads = 3.16) also supported the notion that the persistence of radiation induced damage had been the dominant mechanism determining M_2 phenotype.

Once again there was a downward shift in the distributions of the M_1 and the inbred generations relative to those of the parents and the F_1 (Figure 60). The M_2 also displayed an excess of very low scoring phenotypes. Variances too followed the earlier established pattern, the F_1 being relatively high scoring while variability within M_2 families was low.

- stem colour

The percentage of plants falling into the categories none, little, some and much colour in the stem are presented in Figures 62 to 64. Mean scores (1 being no red, 9 being completely red) are recorded in Tables 49 to 51. The sample size for stem colour and tuber colour differ because the former was recorded at the same time as height was measured when some plants had still to emerge.

As each parent was phenotypically variable for this character, the significance of any genetic component in the expression of stem colour is questionable. In any event, there was little to differentiate the maternal self from the F_2 and the M_2 in any of the crosses.

- tuber colour

By contrast, tuber colour was consistent within the three parental varieties. In over 90% of cases, Pentland Ivory was scored white, Desiree red and Cara intermediate (Figures 65 to 67).

The results for the Pentland Ivory x Cara cross are presented in Figure 65 and Table 49. While the frequency distribution of the M_1 at 5Krad was very similar to that of the F_1 , all 16 M_1 -15Krad plants had no colour in their tubers making them like the maternal parent.

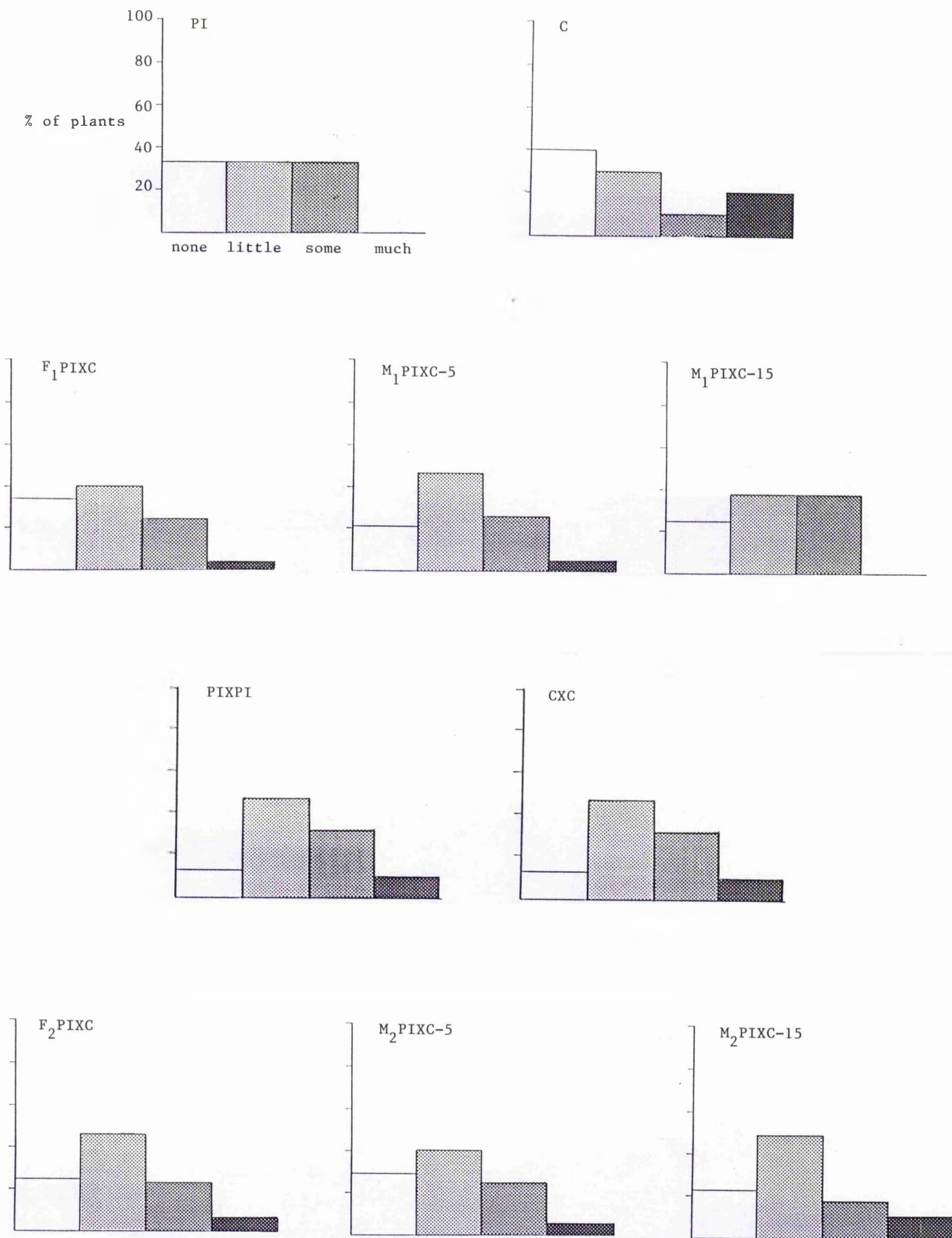


Figure 62: Stem colour distribution for plants in the PIXC cross

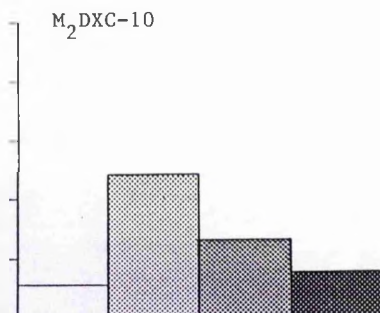
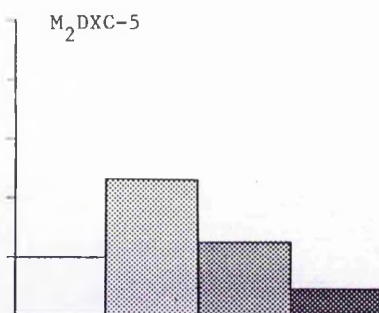
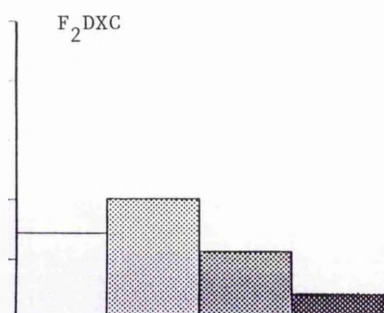
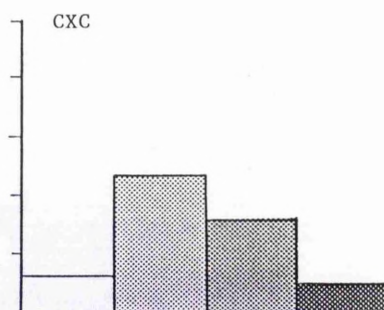
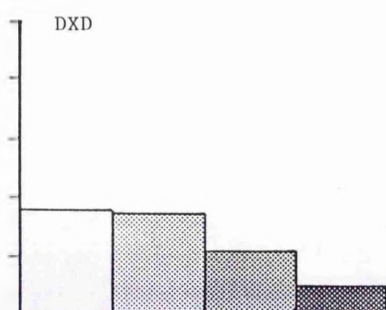
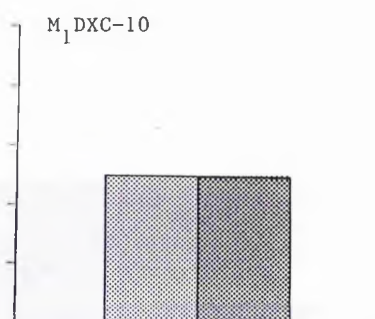
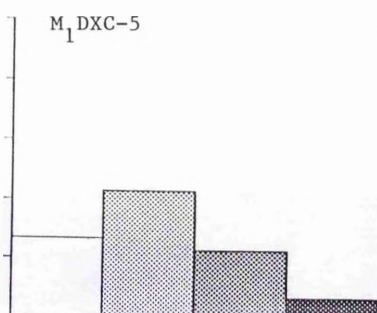
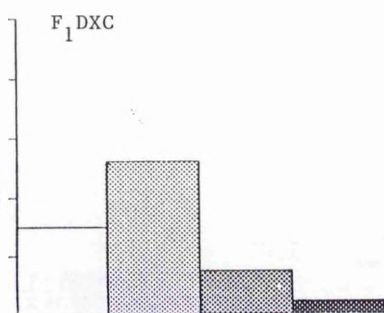
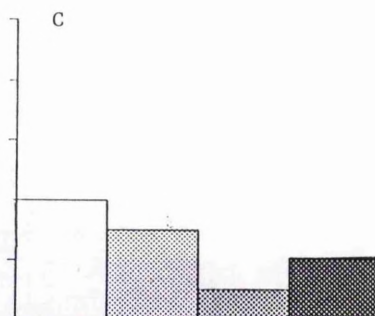
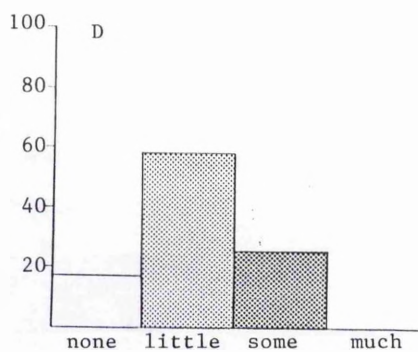


Figure 63: Stem colour distribution for plants in the DXC cross

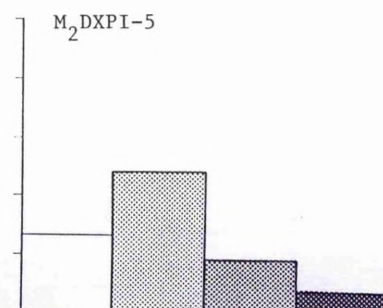
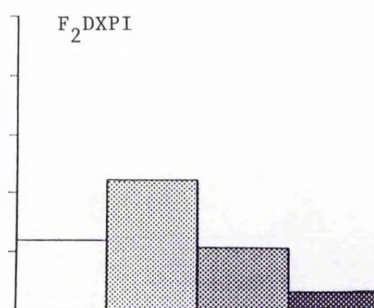
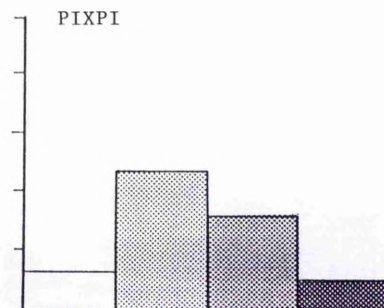
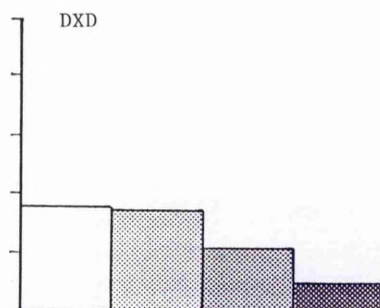
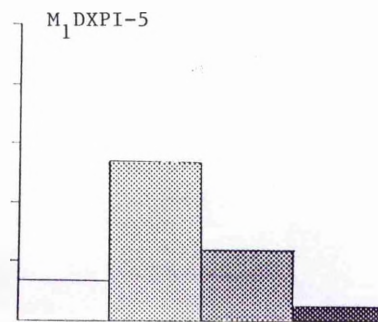
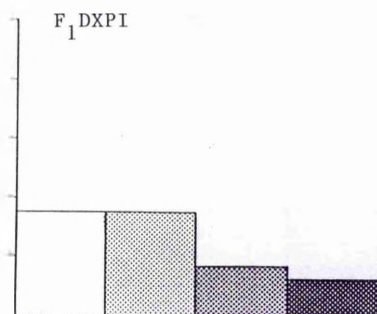
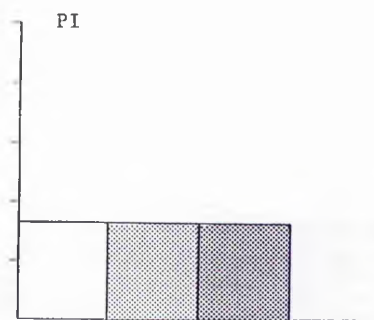
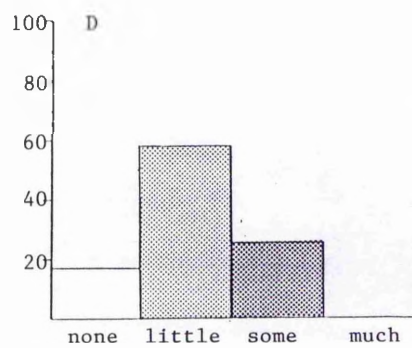


Figure 64: Stem colour distribution for plants in the DXPI cross

	stem colour		tuber colour	
	number of plants	mean score	number of plants	mean score
PI	12	3.33	20	1.25
C	10	3.70	19	3.16
PIxC	49	3.73	89	1.36
CxC	64	4.20	94	2.23
F ₁ PIxC	29	3.24	48	2.46
M ₁ PIxC-5	19	3.68	27	2.30
M ₁ PIxC-15	8	3.63	16	1.00
F ₂ PIxC	346	3.60	585	2.01
M ₂ PIxC-5	64	3.47	96	1.24
M ₂ PIxC-15	82	3.67	106	1.21

Table 49: Mean scores for stem and tuber colour (1 = no red,
9 = red) in the Pentland Ivory x Cara cross

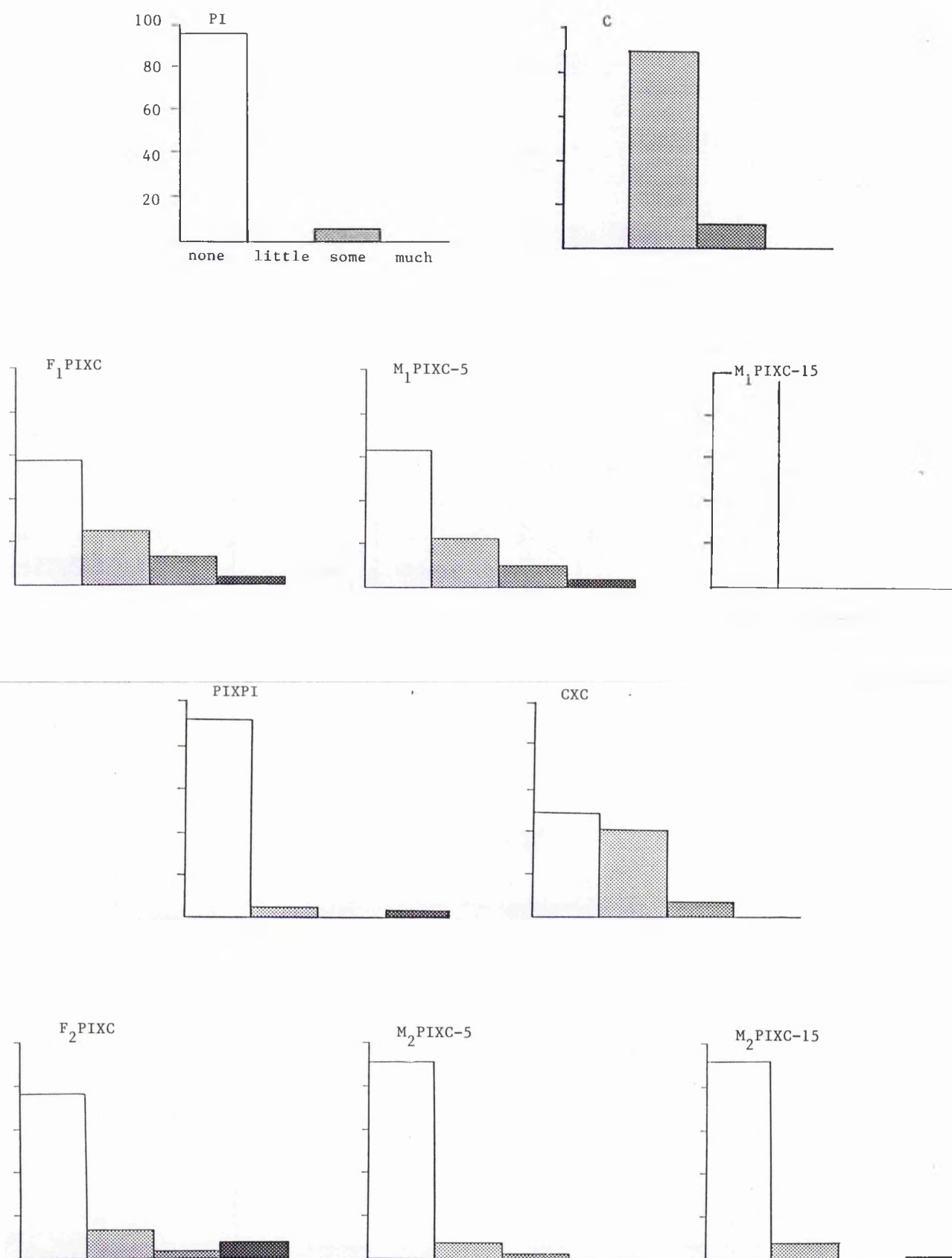


Figure 65: Tuber colour distribution in the PIXC cross

The frequency of F_2 plants without colour in their tubers was slightly lower than in the M_2 . This meant the M_2 (means 1.24 and 1.21) was closer to the maternal self (1.36) than to the F_2 (2.01), although the differences were small.

Figure 66 and Table 50 display the results of the Desiree x Cara cross. The distributions of the F_1 and M_1 for both doses were very similar. In each case just over 40% of tubers had no colour, slightly fewer had much colour, and there were more with a little red than some red. The F_2 (mean 4.46) was more like the maternal self (5.32) than either of the M_2 s (2.80, 2.40).

The results of the third cross, Desiree x Pentland Ivory, are summarised in Figure 67 and Table 51. Again the F_1 and M_1 were similarly distributed with only the relative importance of the two middle classes differing. Indeed, the mean score was identical in both. The same pattern was observed in the second generation. While the F_2 and M_2 closely resembled each other, neither displayed the high proportion of red tubers evident in the maternal self.

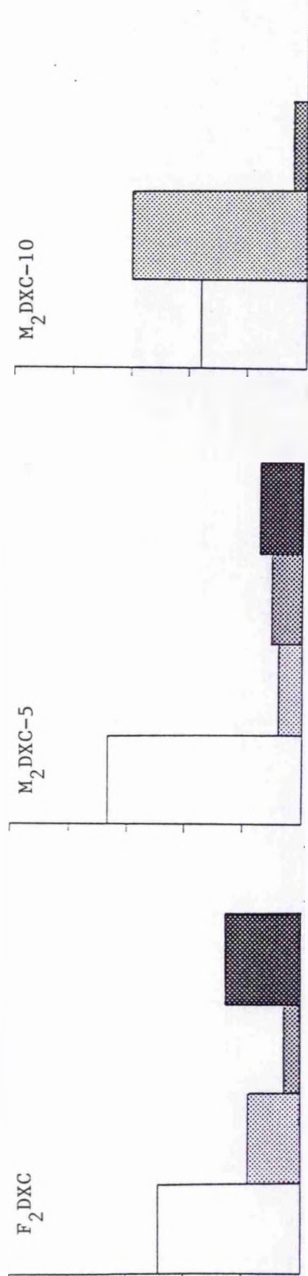
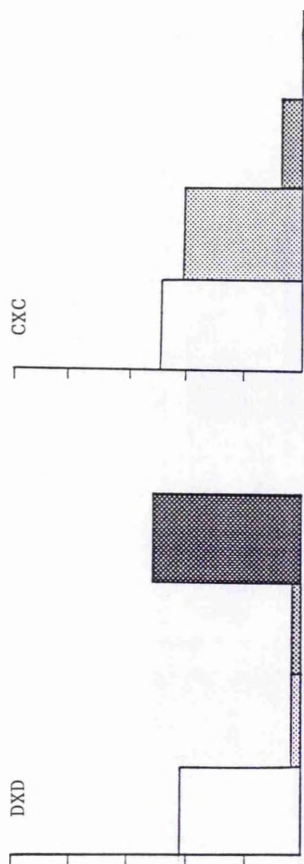
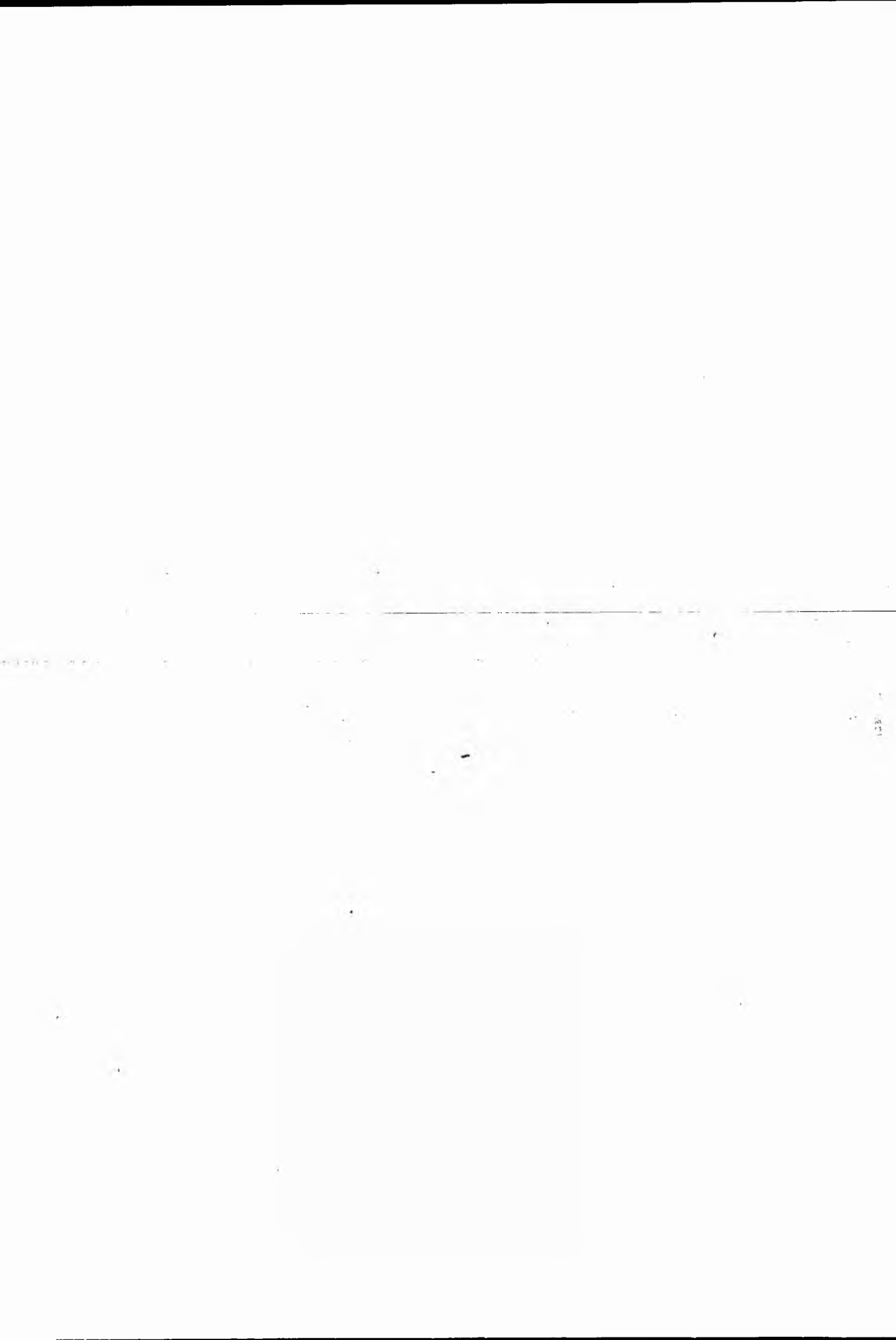
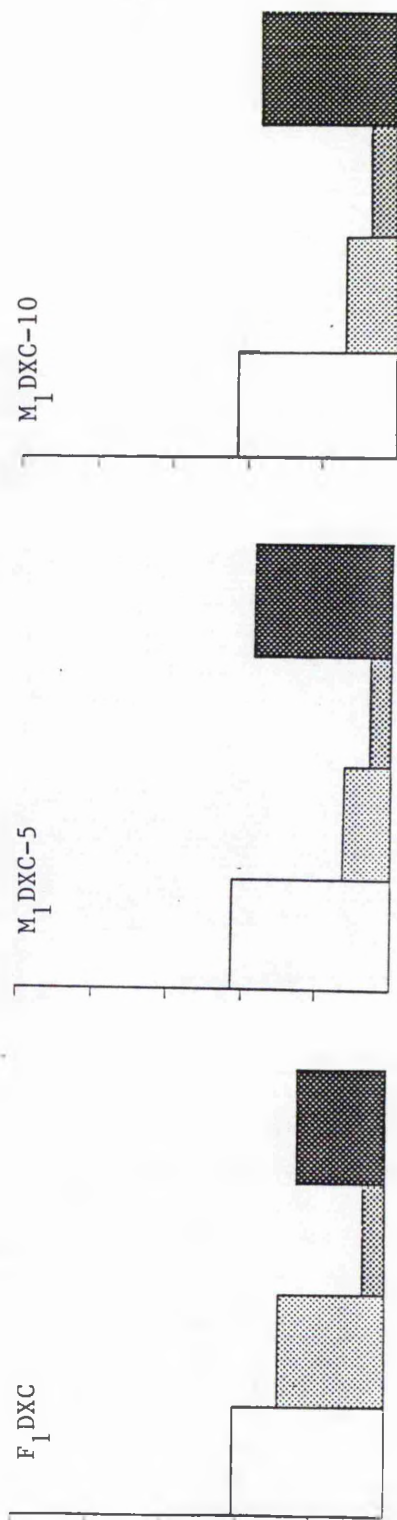
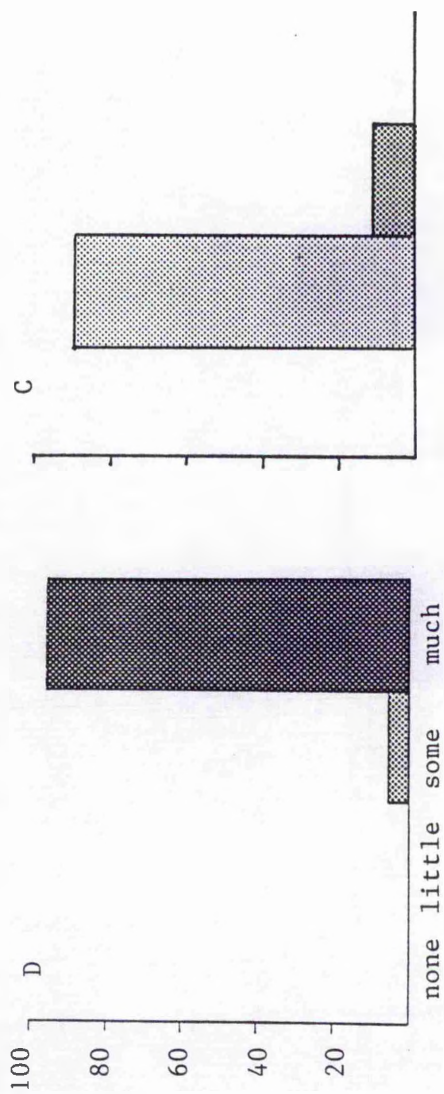


Figure 66: Tuber colour distribution for plants in the DXC cross





	stem colour		tuber colour	
	number of plants	mean score	number of plants	mean score
D	12	3.42	20	8.85
C	10	3.70	19	3.16
DxD	59	3.54	90	5.32
CxC	64	4.20	94	2.23
F ₁ DxC	27	3.07	49	3.84
M ₁ DxC-5	30	3.57	47	4.64
M ₁ DxC-10	10	4.50	14	4.50
F ₂ DxC	181	3.57	253	4.46
M ₂ DxC-5	59	3.86	83	2.80
M ₂ DxC-10	27	4.44	47	2.40

Table 50: Mean scores for stem and tuber colour (1 = no red,
9 = red) in the Desiree x Cara cross

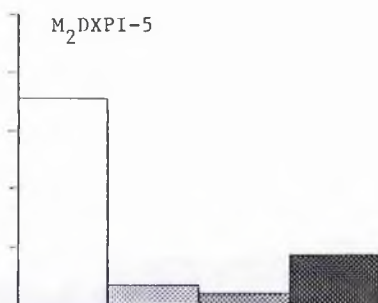
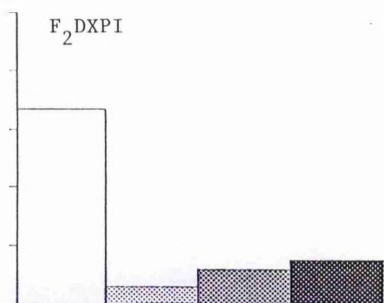
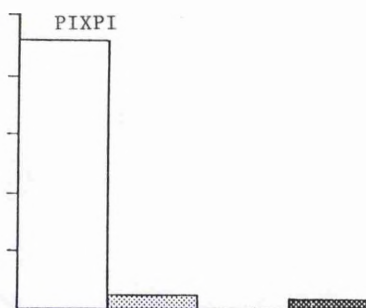
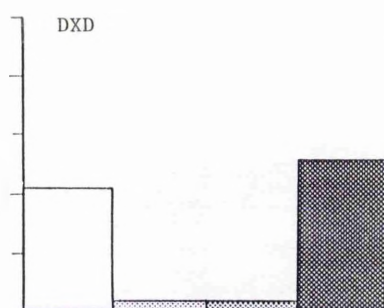
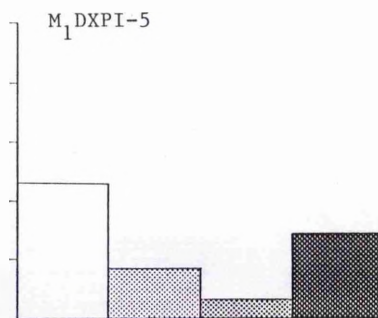
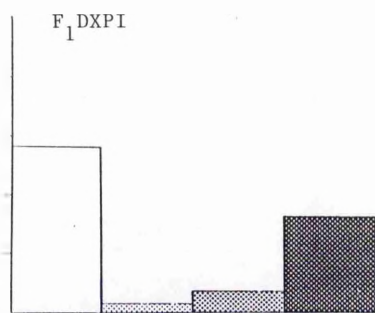
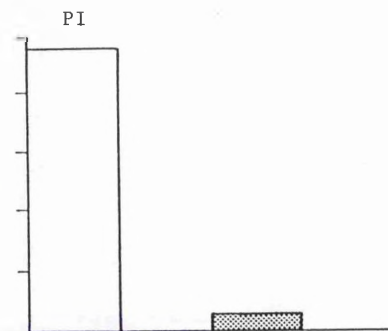
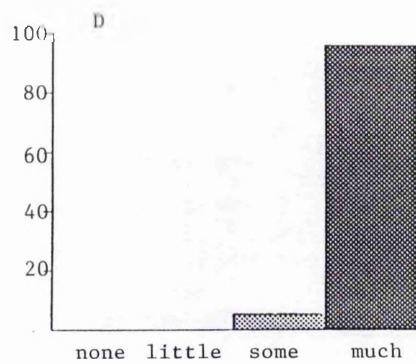


Figure 67: Tuber colour distribution for plants in the DXPI cross

	stem colour		tuber colour	
	number of plants	mean score	number of plants	mean score
D	12	3.42	20	8.85
PI	12	3.33	20	1.25
DxD	59	3.54	90	5.32
PIxPI	49	3.73	94	2.23
F ₁ DxPI	23	3.61	44	4.11
M ₁ DxPI-5	21	3.71	46	4.11
F ₂ DxPI	337	3.45	553	2.78
M ₂ DxPI-5	79	3.46	109	2.89

Table 51: Mean scores for stem and tuber colour (1 = no red, 9 = red) in the Desiree x Pentland Ivory cross

Discussion

For the most part, the proponents of genomic selection and those who have advanced the mutational damage theory have worked on different species. That is, diploid barley on the one side and polyploid Nicotiana on the other. It seems reasonable that the two will differ in the amount of radiation damage they can tolerate. And, therefore, in the amount of damage that will persist after the first generation. That said, it comes as no surprise that pollen irradiation may be a more successful means of limited gene transfer in barley than Nicotiana. In order to see whether the effects of the radiation treatment in the tetraploid potato could provide support for either of the proposed mechanisms, this study was carried out between 1981 and 1985.

- the first generation

In both the irradiated pollen and the irradiated ovule barley studies, the F_1 and the M_1 achieved similar scores for all but seed set. By contrast, the M_1 s in each of the three potato crosses were consistently lower scoring than their F_1 counterparts. Not only were they slower to become established, M_1 individuals were on average shorter and also less productive in terms of both tuber number and tuber weight than those of the F_1 . An increase in the radiation dose, however, was not always associated with a reduction in score. Nevertheless, the consistent shortfall observed in the M_1 suggests radiation induced damage was widespread within this generation.

- the second generation

Although there were not always consistent shifts away from the irradiated parent in the M_2 s of the barley experiments, wherever decreasing trends occurred they were always in the direction of the non-irradiated parent. The experience with potatoes was again different to that in barley. On all but one occasion, the M_2 was lower scoring than the F_2 which was lower or equal scoring to the maternal self.

Although the M_2 deficit was not always significant and there were not consistent dose effects, more often than not the ratios of 1st to 2nd generation scores indicated much damage had persisted. Where it appeared damage had been eliminated, it was of insufficient magnitude to cause a shift towards the maternal self. Moreover, as family variances were always at the lower end of the observed range, M_2 clones were consistently low scoring.

- major genes

As far as the major genes are concerned, little can be drawn from the PVX resistance test results. The pcn tests, on the other hand, revealed a consistent reduction in the frequency of paternal-type, dominant, clones in the M_2 when compared to the F_2 .

Whether the relevant part of the paternal genome had been preferentially eliminated, or it was present but had been inactivated by the radiation treatment, is debatable. But in view of the apparently widespread damage suggested by the quantitative results, it seems reasonable that some inactivation of the dominant resistance gene must have occurred.

- conclusion

Unlike the results of the barley experiment, which although inconclusive provided little support for the mutational damage theory, these findings suggest mutational damage is more important in determining M_2 phenotype than the preferential elimination of the paternal genome when irradiated pollen crosses are carried out in potatoes.

It may well be that this is a characteristic of polyploids, so limiting the value of the technique in such species. But further detailed studies would have to be undertaken to confirm this as the case. Interestingly, pollen irradiation may still have an application in potato breeding. Because it could be of use in cultivated diploid species and, perhaps more importantly, it could be employed in conjunction with dihaploids. Again, only further investigation can confirm or disprove the technique's potential.

CONCLUSION

Traditionally, plant breeding has involved the combination of whole genomes, the later segregation of characters and the selection of plants over a number of following generations [Hadley & Openshaw, 1980]. As Davies [1981] acknowledged, when the breeding objective is the transfer of just a single characteristic from one genotype to another, this method is both lengthy and laborious. Moreover, the end result is not guaranteed and even when successful it can take many generations to achieve. But it was not until quite recently that any alternative method of facilitating limited gene transfer emerged.

Naturally, the advent of nucleic acid manipulation was greeted with much optimism. But while the potential of genetic engineering techniques remains high, they have yet to become routinely available to plant breeders [Borlaug, 1983]. What's more, the number of species in which such methods have so far been applied is very small. Understandably then, the early reports of limited gene transfer following irradiated pollen crosses [Pandey 1975; Virk et al., 1977, Jinks et al., 1981] stimulated several other workers to experiment with the technique. Because pollen irradiation utilised conventional practices, breeders were largely familiar with the general protocols. And, since the only additional requirement was the facility to irradiate samples, the technique was relatively cheap and easy.

Two schools of thought as to the value of pollen irradiation soon emerged. There were those who believed that the observed maternal trends could be explained largely in terms of radiation induced mutational damage [eg Werner et al., 1984]. While others [eg Snape et al., 1983; Powell et al.; 1983; Davies 1984] suggested that preferential elimination of the paternal genome during the formation of the second generation might be responsible. Since the value of the technique depended on the extent to which mutational damage persisted, experiments were set up with the aim of clarifying which mechanism was involved.

Cytological investigations proved inconclusive. Snape and co-workers [1983] found that while most M_1 wheat plants exhibited aneuploidy and signs of structural damage, by the M_2 karyotypes appeared much more normal. Yet in Nicotiana rustica [Werner & Cornish, 1984], the frequency at which aneuploidy and deletions persisted into the M_2 generation was high. The findings in barley [Borrino et al., 1985], the only diploid species of the three, were different again. Only a small percentage of M_1 plants showed evidence of structural rearrangements, and the percentage was even smaller in the M_2 . Unfortunately, the situation was little clearer when whole plants were measured.

If, as Werner and Cornish [1985] suspected, mutational damage was dominant in determining M_2 phenotype, then some sort of variability would be expected in the second generation following selfing with irradiated pollen. But if genomic selection was largely responsible, it ought to be possible to demonstrate opposite trends in reciprocal irradiated crosses, parental differences permitting.

Most of the evidence in the mechanism debate has come from studies carried out in barley and Nicotiana. Powell and Caligari [1985] could find no significant differences between irradiated and control barley selfs. So they concluded that, at least in barley, damage induced by irradiated pollen could not adequately account for maternal trends observed in later generations.

Unfortunately, when Cornish and Werner [1985] carried out reciprocal irradiated pollen crosses in Nicotiana, the consistent trends were for reduced vigour rather than towards the maternal parent. And while disturbed segregations of major genes were observed in favour of maternal alleles, the authors stated that such effects were slight and only achieved at the expense of considerable deleterious radiation damage. However, Werner and Cornish did not explain why irradiated selfs in the same experiment closely resembled their respective parents. Clearly, before pollen irradiation could be recommended or totally dismissed, further investigations were necessary.

Since the results in barley had been most promising, and in the absence of successful genetic engineering techniques in the species, reciprocal irradiated crosses formed the first part of this investigation. While the findings of the study clearly showed that considerable deleterious radiation damage was not present in the M_2 , unfortunately maternal trends were not consistently demonstrated either!

More promising results were obtained in the barley irradiated ovule experiment. Since the mechanism dispute concerns what happens during the formation of the second generation, it seems feasible that similar processes will come into play whichever gamete is irradiated. So consistent trends for reduced vigour would be expected if widespread damage persisted in the M_2 , while trends to the paternal parent would occur if genomic selection predominated.

As in the irradiated pollen study, there was no evidence to suggest that mutational damage had played a large part in determining M_2 phenotype. In fact, whenever shifts from the F_2 occurred, they were for increased vigour. What's more, an excess of paternal alleles was observed in the M_2 for all qualitative traits although its magnitude varied from character to character.

By contrast, the results of irradiated crosses in the tetraploid potato suggested significant mutational damage was present in both the M_1 and M_2 generations. Since these results are similar to those obtained by Werner and Cornish in Nicotiana, it may be that this feature is characteristic of polyploid crops subjected to irradiated pollen crossing.

In view of the conflicting results, it is difficult to predict any benefits of gamete irradiation over backcrossing in different crops on the basis of this study. However, in diploid species such as barley, ovule irradiation may not only be more effective than its male equivalent, it may also be easier to carry out.

As in the evaluation of pollen irradiation, reciprocal crosses and irradiated ovule selfs need to be performed to establish the efficacy of the technique. Moreover, only experience with different crops will tell if the method can be widely applied. Both types of gamete irradiation may be useful in breaking down linkages that resist conventional crossing.

As far as polyploid crops are concerned, it seems that gamete irradiation is unlikely to be a useful tool because of the persistence of deleterious radiation effects. That said, when the female rather than the male gamete is irradiated, the results might be more promising. Moreover, in crops such as the potato where reduced forms (e.g. dihaploids) are available, radiation damage is less likely to be tolerated and so the technique may achieve some success.

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APPENDICES

Table A1: Analysis of variance of data from the F_2 / M_2 generations in the barley irradiated pollen study

Key:

= non-significant

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.005$

[For each parent, any 'within families' variation must have been environmental in origin since individuals were genetically identical. So the results for both parents were pooled to estimate the environmental mean square for each character. Each within families mean square was tested against its environmental mean square to confirm that there were genetic differences among individuals for each trait.

Since both main effects (families, reps) in this analysis were random, the appropriate comparison for the between families mean square (MS) was, where it was significant, the interaction MS. Where it was not significant, the interaction MS provided an estimate of error that could be pooled with the within families MS and used to test the significance of the between family MS.]

GP	within families		
	\bar{x}	MS	df
height	57.33	26.281	106
tiller number	5.42	8.609	106
ear length	7.78	0.714	106
awn length	12.39	0.948	97
green tiller number	2.41	3.038	106

between families		error		VR	P
MS	df	MS	df		
291.5184	2	97.4030	2	2.99	
25.5485	2	8.5492	108	2.99	
1.6199	2	4.3260	2	0.37	
5.5396	2	6.1327	2	0.90	
95.9805	2	45.2932	2	2.12	

S138

within families

	\bar{x}	MS	df
height	64.80	54.982	30
tiller number	4.49	11.746	30
ear length	6.19	1.191	30
awn length	15.39	2.320	30
green tiller number	1.47	3.577	30

between families		error		VR	P
MS	df	MS	df		
59.2900	2	52.4557	32	1.13	
7.8671	2	11.0550	32	0.71	
0.0406	2	1.1281	32	0.03	
9.9276	2	2.4939	32	3.98	*
1.8267	2	3.8890	32	0.47	

F ₂ GP x S138	\bar{x}	within families		environment		VR
		MS	df	MS	df	
height	68.26	86.409	336	32.612	136	2.59
tiller number	5.87	17.668	336	9.301	136	1.90
ear length	7.30	1.495	336	0.819	136	1.83
awn length	14.69	6.272	287	1.272	127	4.93
green tiller number	1.34	3.655	336	3.157	136	1.16

between
families

error

P	MS	df	MS	df	VR	P
***	592.7781	9	86.3627	345	6.86	***
***	44.7579	9	17.8471	345	2.51	**
***	10.6987	9	1.4946	345	7.16	***
***	21.1327	9	13.9413	9	1.52	
	9.3598	9	3.7272	345	2.51	**

		within families		environment		
M2GP x S138 -500rads	x	MS	df	MS	df	VR
height	68.62	92.920	680	32.612	136	2.85
tiller number	6.36	14.170	680	9.301	136	1.52
ear length	7.57	1.582	680	0.819	136	1.93
awn length	14.83	6.448	615	1.272	127	5.07
green tiller number	1.50	3.575	680	3.157	136	1.13

between
families

error

P	MS	df	MS	df	VR	P
***	209.1058	24	92.6452	704	2.26	***
***	36.3253	24	31.1520	24	1.17	
***	3.0211	24	1.5886	704	1.90	***
***	18.2524	24	22.3280	24	0.82	
	6.7845	24	10.1414	24	0.67	

		within families		environment		
M ₂ GP x S138 -1000rads	x	MS	df	MS	df	VR
height	66.92	86.222	372	32.612	136	2.64
tiller number	7.19	26.687	372	9.301	136	2.87
ear length	7.45	1.498	372	0.819	136	1.83
awn length	15.26	6.396	323	1.272	127	5.03
green tiller number	1.78	4.241	372	3.157	136	1.34

between families			error		VR	P
P	MS	df	MS	df		
***	300.2012	11	87.852	383	3.42	***
***	51.5746	11	26.7719	383	1.93	*
***	1.7178	11	1.4945	383	1.15	
***	35.2301	11	15.2122	11	2.32	
*	20.6329	11	4.2534	383	4.85	***

	within families			environment			between families			error		
	\bar{x}	MS	df	MS	df	VR	P	MS	df	MS	df	P
M2GP x S138 -1500rads												
height	67.08	125.510	27	32.612	136	3.85	***	-	-	-	-	-
tiller number	6.08	23.654	27	9.301	136	2.54	***	-	-	-	-	-
ear length	7.67	1.491	27	0.819	136	1.82	*	-	-	-	-	-
awn length	16.56	5.141	24	1.272	127	4.04	***	-	-	-	-	-
green tiller number	2.73	5.820	27	3.157	136	1.84	*	-	-	-	-	-

F ₂ S138 x GP	within families			environmental		
	\bar{x}	MS	df	MS	df	VR
height	68.93	78.311	318	32.612	136	2.40
tiller number	6.17	17.631	318	9.301	136	1.90
ear length	7.54	1.281	318	0.819	136	1.56
awn length	15.14	4.865	275	1.272	127	3.82
green tiller number	1.30	3.263	318	3.157	136	1.03

between families			error			
P	MS	df	MS	df	VR	P
***	65.3437	9	78.4202	327	0.83	
***	30.3133	9	17.5072	327	1.73	
**	0.6179	9	1.2906	327	0.48	
***	20.9669	9	15.1172	9	1.39	
	4.0794	9	3.2937	327	1.24	

		within families		environment		
M ₂ S138 x GP -500 rads	x	MS	df	MS	df	VR
height	68.52	94.990	545	32.612	136	2.91
tiller number	6.32	22.285	545	9.301	136	2.40
ear length	7.30	1.393	545	0.819	136	1.70
awn length	14.82	5.815	477	1.272	127	4.57
green tiller number	1.42	3.890	545	3.157	136	1.23

between families			error			
P	MS	df	MS	df	VR	P
***	404.6364	17	205.5407	17	1.97	
***	24.7653	17	22.7297	562	1.09	
***	4.4394	17	1.4014	562	3.17	***
***	20.1817	17	12.8481	17	1.57	
	7.5978	17	3.9326	562	1.93	*

		within families		environment		
M ₂ S138 x GP -1000rads	x	MS	df	MS	df	VR
height	67.28	95.126	207	32.612	136	2.92
tiller number	6.57	23.156	207	9.301	136	2.49
ear length	7.46	1.178	207	0.819	136	1.44
awn length	15.09	5.754	173	1.272	127	4.52
green tiller number	1.57	3.819	207	3.157	136	1.21

between
families

error

P	MS	df	MS	df	VR	P
***	430.5477	6	95.4225	213	4.51	***
***	10.1327	6	23.1543	213	0.44	
*	1.8319	6	2.6260	6	0.70	
***	15.8086	6	23.8878	6	0.66	
	13.7763	6	14.4910	6	0.95	

**Table A2: Major gene frequencies in the second generation of the barley
irradiated pollen study**

Gene		Frequency	
Hv-1		0.000	
Hv-2		0.000	
Hv-3		0.000	
Hv-4		0.000	
Hv-5		0.000	
Hv-6		0.000	
Hv-7		0.000	
Hv-8		0.000	
Hv-9		0.000	
Hv-10		0.000	
Hv-11		0.000	
Hv-12		0.000	
Hv-13		0.000	
Hv-14		0.000	
Hv-15		0.000	
Hv-16		0.000	
Hv-17		0.000	
Hv-18		0.000	
Hv-19		0.000	
Hv-20		0.000	
Hv-21		0.000	
Hv-22		0.000	
Hv-23		0.000	
Hv-24		0.000	
Hv-25		0.000	
Hv-26		0.000	
Hv-27		0.000	
Hv-28		0.000	
Hv-29		0.000	
Hv-30		0.000	
Hv-31		0.000	
Hv-32		0.000	
Hv-33		0.000	
Hv-34		0.000	
Hv-35		0.000	
Hv-36		0.000	
Hv-37		0.000	
Hv-38		0.000	
Hv-39		0.000	
Hv-40		0.000	
Hv-41		0.000	
Hv-42		0.000	
Hv-43		0.000	
Hv-44		0.000	
Hv-45		0.000	
Hv-46		0.000	
Hv-47		0.000	
Hv-48		0.000	
Hv-49		0.000	
Hv-50		0.000	
Hv-51		0.000	
Hv-52		0.000	
Hv-53		0.000	
Hv-54		0.000	
Hv-55		0.000	
Hv-56		0.000	
Hv-57		0.000	
Hv-58		0.000	
Hv-59		0.000	
Hv-60		0.000	
Hv-61		0.000	
Hv-62		0.000	
Hv-63		0.000	
Hv-64		0.000	
Hv-65		0.000	
Hv-66		0.000	
Hv-67		0.000	
Hv-68		0.000	
Hv-69		0.000	
Hv-70		0.000	
Hv-71		0.000	
Hv-72		0.000	
Hv-73		0.000	
Hv-74		0.000	
Hv-75		0.000	
Hv-76		0.000	
Hv-77		0.000	
Hv-78		0.000	
Hv-79		0.000	
Hv-80		0.000	
Hv-81		0.000	
Hv-82		0.000	
Hv-83		0.000	
Hv-84		0.000	
Hv-85		0.000	
Hv-86		0.000	
Hv-87		0.000	
Hv-88		0.000	
Hv-89		0.000	
Hv-90		0.000	
Hv-91		0.000	
Hv-92		0.000	
Hv-93		0.000	
Hv-94		0.000	
Hv-95		0.000	
Hv-96		0.000	
Hv-97		0.000	
Hv-98		0.000	
Hv-99		0.000	
Hv-100		0.000	

		RSert	RSert	RsErt	rSErt	Rsert	rSert	rsErt	rsert
F2GPxS138	1	19	6	3	3	1	-	6	-
	2	21	1	4	3	-	2	3	-
	3	10	5	4	2	1	1	8	1
	4	20	6	3	1	1	1	4	1
	5	10	11	3	4	-	1	3	1
	6	15	4	5	3	-	-	4	1
	7	12	10	7	-	1	3	3	1
	8	20	5	5	4	1	-	2	1
	9	19	8	6	1	-	1	1	1
	10	17	5	2	3	1	-	4	2
F2S138xGP	1	12	8	2	6	1	3	5	-
	2	17	3	1	3	-	2	7	1
	3	10	7	2	4	-	-	5	1
	4	16	7	3	-	-	1	5	-
	5	14	6	7	2	-	1	3	1
	6	13	7	2	1	1	-	4	-
	7	15	3	2	7	-	1	8	1
	8	20	10	6	1	-	1	1	-
	9	8	10	2	3	-	1	7	1
	10	14	8	3	2	-	1	3	2
M2GPxS138 -500rads	1	21	7	2	-	-	1	2	2
	2	18	4	-	6	-	-	3	-
	3	21	5	1	-	1	-	4	-
	4	13	11	2	2	-	2	2	-
	5	13	6	3	2	-	1	9	-
	6	18	4	2	3	-	1	5	-
	7	3	6	1	2	-	1	1	-
	8	6	1	-	-	-	-	3	-
	9	19	4	4	4	-	-	3	-
	10	17	9	3	3	-	2	1	-
	11	7	4	2	3	1	1	1	-
	12	14	2	4	1	1	1	-	1
	13	19	6	4	5	-	-	3	1
	14	13	1	1	1	-	-	3	1
	15	10	3	1	2	-	-	4	-
	16	12	2	1	5	-	-	6	1
	17	11	6	3	6	-	1	2	-
	18	14	10	3	1	-	1	5	-
	19	9	4	2	2	-	-	1	-
	20	13	4	4	1	1	1	8	-
	21	15	7	3	4	-	4	1	-
	22	19	7	2	4	-	-	2	2
	23	10	7	2	1	-	1	8	-
	24	11	8	1	3	-	1	4	1
	25	11	6	5	2	-	3	3	1

		RSert	RSert	RsErt	rSErt	Rsert	rSert	rsErt	rsert
M ₂ GPxSl38 -1000rads	1	19	10	-	1	-	-	3	-
	2	15	14	2	-	2	1	1	1
	3	20	10	2	-	-	2	2	1
	4	28	2	1	4	-	-	1	-
	5	13	8	3	-	2	-	7	-
	6	12	9	4	-	-	-	-	-
	7	13	7	3	-	-	-	3	-
	8	11	11	-	3	-	1	3	-
	9	12	8	1	3	2	1	5	-
	10	13	7	3	4	1	2	3	-
	11	18	3	6	-	-	1	5	-
	12	13	8	1	-	-	1	-	-

M ₂ Sl38xGP -500rads	1	14	3	1	5	-	1	1	-
	2	14	5	3	2	-	-	1	1
	3	17	4	6	3	-	1	5	-
	4	22	7	3	3	1	-	3	-
	5	10	9	3	1	-	-	1	-
	6	16	6	5	2	-	1	5	-
	7	21	7	2	-	-	1	2	-
	8	22	5	4	-	-	1	-	-
	9	18	6	6	3	1	-	2	-
	10	10	9	2	2	1	1	5	2
	11	19	6	3	2	-	2	4	-
	12	9	7	1	1	-	-	5	-
	13	12	6	4	3	1	-	1	1
	14	15	5	3	4	1	-	4	1
	15	17	7	5	1	-	1	3	-
	16	11	5	5	2	-	2	2	-
	17	14	6	-	1	-	2	5	-
	18	19	8	4	-	-	2	3	-

M ₂ GPxSl38 -1000rads	1	18	6	5	2	1	2	1	-
	2	18	3	-	3	1	1	4	-
	3	15	8	2	4	-	1	4	-
	4	4	2	2	3	1	3	1	-
	5	21	5	5	2	-	1	2	-
	6	15	0	2	3	-	1	2	-
	7	20	6	2	1	-	2	3	-

Table A3: χ^2 analysis of M_2 families whose major gene frequencies differed significantly from those of the F_2 in the barley irradiated pollen study

Key:

= non-significant

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.005$

generation (family)	R:r	chi ² P	S:s	chi ² P	Ert:ert	chi ² P
pooled F2	508:177		514:171		534:162	

M2 GPxS138
-500rads

(1)			29:6	6.38*	19:13	5.36*
(4)						
(16)	15:12	4.89*	19:13	4.17*		
(20)			556:154	4.06*		
(pooled)						

-1000rads

(2)	33:3	4.88*	34:2	6.24*	18:18	14.06***
(4)					34:2	5.67*
(6)	30:0	9.14***				
(8)					17:12	5.97*
(12)	22:1	4.47*	22:1	4.17*		

M2 S138xGP
-500rads

(7)	30:3	4.00*				
(8)	31:1	7.47**			19:13	5.36*
(10)						
(pooled)	455:106	14.12***				

-1000rads

178:38 6.26*

	RS:Rs:rS:rs	chi ²	df	P	Rert:Rert:rErt:rert	chi ²	df	P	Sert:Sert:sErt:sert	chi ²	df	P
pooled F ₂	432:80:73:100				374:138:139:34				355:150:168:22			

M₂ GPxS138
-500rads

(2)					13:2:11:1	8.03	3	*	24:4:3:0	8.69	2	*
(16)												
(21)	22:3:8:1	8.64	3	*								
(pooled)	471:60:85:94	9.56	3	*					400:156:140:14	12.92	3	***

-1000rads

(1)	19:0:1:3	9.57	3	*								
(2)					17:16:1:2	16.39	3	*	15:15:3:3	14.12	3	***
(4)	30:1:4:1	8.24	3	*	29:2:5:0	10.75	3	*	32:2:2:0	20.66	3	***
(6)	26:4:0:0	10.30	3	*	16:9:0:0	9.83	3	*				
(12)	21:1:1:0	8.19	3	*								

M₂ S138xGP

-500rads

(8)	27:4:1:0	8.70	3	*	26:5:0:1	11.20	3	*	24:4:3:0	8.69	2	*
(pooled)	391:64:49:57	13.51	3	***	340:114:85:20	13.27	3	***	315:126:112:9	11.43	3	***

-1000rads

(7)	26:2:3:3	13.88	3	***								
(pooled)	149:21:29:17	10.16	3	*					129:51:35:3	11.28	3	*

**Table A4: Correlation analysis of the second generation data from the
the barley irradiated pollen study**

Key:

= non-significant

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.005$

To test the hypothesis that all r s are estimates of the same ρ :-

$$\chi^2 = \frac{\sum (n_i - 3) Z_i^2 - \left[\sum (n_i - 3) Z_i \right]^2}{\sum (n_i - 3)}$$

To combine independent estimates of ρ :-

$$Z_{(\rho)} = \sum (n_i - 3) Z_i / \sum (n_i - 3)$$

To test the significance of the difference between r and ρ :-

$$F = (Z_{(r)} - Z_{(\rho)})^2 / \sigma^2_{(Z)}$$

$$\sigma^2_{(Z)} = 1/(n-3)$$

F ₂ GPxSL38		height & tiller number			height & ear length			tiller number & ear length		
	n	Z	F	P	Z	F	P	Z	F	P
family 1	37	.3972	-	-	1.0153	-	-	.5967	-	-
2	34	.2686	-	-	.5563	-	-	.4199	-	-
3	32	.2029	-	-	.3959	-	-	.3350	-	-
4	38	.2605	-	-	.4805	-	-	.4933	-	-
5	33	.4252	-	-	.7162	-	-	.5855	-	-
6	36	.1478	-	-	.6543	-	-	.5908	-	-
7	37	.5439	-	-	.7612	-	-	.4526	-	-
8	38	.0050	-	-	.8753	-	-	.2734	-	-
9	38	.1348	-	-	.6511	-	-	.2869	-	-
10	33	.0589	-	-	.4247	-	-	.3452	-	-

$\chi^2 = 8.67$
 $P = 0.4685$

$\chi^2 = 11.51$
 $P = 0.2426$

$\chi^2 = 4.72$
 $P = 0.8580$

M₂ GPXSI38

-500rads

height & tiller number

height & ear length

tiller number & ear length

n Z F P

Z F P

Z F P

family

1 35

-.2500

7.87

**

.2537

3.91

*

.5417

0.26

2

30

.4481

1.10

.8521

1.67

.3948

0.09

3

33

.6609

5.17

*

.8613

2.00

.7237

2.21

4

31

-.1695

4.83

*

.2951

2.66

-.2558

14.04

5

36

.1004

0.70

.6216

0.01

.6308

1.06

6

33

.1827

0.12

.4448

0.75

.3964

0.10

7

22

.6486

3.08

.6751

0.10

.7015

1.18

8

11

.3234

0.05

.8444

0.47

.6944

0.47

9

34

.2891

0.06

.4352

0.86

.6578

1.31

10

34

.4768

1.65

.4354

0.87

.2382

1.42

11

19

.2967

0.04

.4857

0.22

.2266

0.82

12

25

-.3097

6.79

**

.3966

0.94

.0615

3.36

M ₂ GPxSI38 -500rads		height & tiller number			height & ear length			tiller number & ear length		
family	n	z	F	P	z	F	P	z	F	P
13	38	.0250	1.71		.7643	0.91		.2703	1.16	
14	19	.2660	0.00		.6791	0.09		.6623	0.71	
15	20	-.3056	5.17	*	.0376	5.45	*	.6752	0.84	
16	28	.5404	2.17		.7692	0.69		.6403	0.88	
17	27	.1110	0.44		-.1543	13.77	***	.2507	0.98	
18	34	.3349	0.25		.3656	1.75		.8093	3.95	*
19	18	-.2736	4.05	*	-.1902	9.44	***	.5037	0.04	
20	30	.0652	0.88		.8271	0.85		.4402	0.00	
21	36	.0648	1.08		.4158	1.16		.5606	0.39	
22	36	.2374	0.00		.4115	1.21		.1904	2.26	
23	36	.3629	0.45		.6131	0.00		.6414	1.18	
24	29	-.0704	2.60		.2894	2.56		.4364	0.01	
25	31	.2063	0.04		.4198	0.74		.4720	0.01	

chi² = 47.16
P = 0.0032***

chi² = 43.09
P = 0.0097***

chi² = 38.75
P = 0.0289*

M ₂ GPxSl38 -1000rads		height & tiller number			height & ear length			tiller number & ear length		
family	n	Z	F	P	Z	F	P	Z	F	P
1	33	.3623	0.41		.7176	0.39		1.0439	10.50	***
2	36	.3697	0.51		.5101	0.29		.4401	0.00	
3	39	.5912	4.29	*	1.0150	6.10	*	.6033	0.82	
4	35	.1194	0.51		.3760	1.65		.6189	0.89	
5	36	-.0157	2.26		.4122	1.21		.4783	0.02	
6	30	.0982	0.59		.2218	3.93	*	.7612	2.58	
7	30	.2890	0.05		.5771	0.02		.5083	0.08	
8	29	.2150	0.02		.7995	1.00		.3471	0.29	
9	36	.3433	0.31		.6707	0.15		.4237	0.03	
10	34	.4115	0.85		.7756	0.92		.5976	0.65	
11	32	.1594	0.22		.7541	0.66		.3037	0.64	
12	23	.5718	2.12		1.0688	4.33	*	.5962	0.41	

chi² = 12.24

P = 0.3456

chi² = 19.85

P = 0.0475*

chi² = 12.83

P = 0.3046

F ₂ S138xGP		height & tiller number				height & ear length				tiller number & ear length			
		n	Z	F	P	Z	F	P	Z	F	P		
family	1	37	-.0273	-	-	.4873	-	-	.2763	-	-		
	2	33	.1809	-	-	.7598	-	-	.1741	-	-		
	3	29	.0735	-	-	.6121	-	-	.3942	-	-		
	4	33	.2857	-	-	.5508	-	-	.5589	-	-		
	5	35	.1539	-	-	.5061	-	-	.3459	-	-		
	6	28	.1983	-	-	.7549	-	-	.4740	-	-		
	7	37	.3891	-	-	.5288	-	-	.6828	-	-		
	8	39	.4367	-	-	.5564	-	-	.6660	-	-		
	9	34	.5439	-	-	.4499	-	-	.5790	-	-		
	10	33	.2292	-	-	.2700	-	-	.4020	-	-		

chi² = 8.57
P = 0.4778

chi² = 8.34
P = 0.4999

chi² = 8.18
P = 0.5159

M ₂ S138xGP -500rads		height & tiller number			height & ear length			tiller number & ear length		
	n	Z	F	P	Z	F	P	Z	F	P
family 1	25	-.1765	7.55	**	.1572	2.00		.5370	0.06	
2	26	-.0081	4.01	*	.7217	1.59		.1927	3.60	
3	36	.1515	2.19		.5880	0.55		.6111	0.02	
4	38	-.2558	15.49	***	.6453	1.22		.0214	11.25	***
5	26	.2347	0.70		.4518	0.00		.1716	3.99	*
6	35	.4341	0.02		.4038	0.10		.5099	0.20	
7	33	.3080	0.31		.8599	4.83	*	.5168	0.15	
8	32	-.0241	5.45	*	.6460	1.02		.1429	5.75	*
9	38	-.0397	7.06	**	.4318	0.03		.4767	0.44	
10	32	.2692	0.57		.5961	0.55		.5104	0.18	
11	36	.2279	1.09		.6882	1.74		.2921	2.90	
12	24	.0301	3.02		.9554	5.18	*	.5001	0.16	

M ₂ SL38xGP -500rads	height & tiller number			height & ear length			tiller number & ear length			
	n	z	F	P	z	F	P	z	F	P
family	13	30	.4066	0.00		.7138	1.76		.8258	1.52
	14	32	-.1849	10.24	***	.5271	1.98		.3065	2.30
	15	33	.2322	0.94		.5960	0.57		.3596	1.57
	16	34	.0815	3.33		.5204	0.12		.3206	2.22
	17	32	-.3651	17.40	***	.4509	0.00		.0060	9.83
	18	34	-.0633	3.71		.1040	3.90	*	-.2861	23.70

chi² = 26.44
P = 0.0668

chi² = 20.31
P = 0.2587

chi² = 34.83
P = 0.0065***

M ₂ sl38xGP -1000rads	height & tiller number				height & ear length				tiller number & ear length			
	n	Z	F	P	Z	F	P	Z	F	P		
family 1	36	.3178	0.28		.7034	1.98		.4707	0.47			
2	32	.0893	2.97		.4121	0.06		.5710	0.01			
3	34	.2909	0.44		.2782	1.01		.2265	4.06	*		
4	16	.7816	1.80		.8919	2.44		.6208	0.01			
5	34	.0481	4.05	*	.4137	0.06		.4568	0.54			
6	34	.4634	0.09		.4947	0.04		.5691	0.01			
7	34	.4868	0.19		.3428	0.42		.5751	0.01			

chi² = 8.13
P = 0.3212

chi² = 5.97
P = 0.4270

chi² = 3.03
P = 0.8054

**Table A5: Results of 't' tests for M₂ families in the barley irradiated
pollen study**

Key:

= non-significant

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.005$

Rough vs smooth awns: M2 GPxSl38-500rads

	Height t & p		Tiller No t & p		Ear Length t & p	
	rep 1	rep 2	rep 1	rep 2	rep 1	rep 2
1.	+0.501	+0.275	-0.150	+0.557	+0.626	-0.180
2.	-0.479	-0.902	-0.201	+0.195	+0.477	-0.845
3.	-1.034	-1.388	+0.265	-0.703	-1.619	-0.643
4.	+0.277	-0.046	+1.029	+0.375	+0.326	+0.999
5.	+0.617	-0.933	+0.805	+0.099	+0.777	-0.872
6.	-0.430	+1.145	+0.112	-0.595	-1.116	+0.129
7.	-0.962	-	-2.669*	-	-1.431	-
8.	-0.708	-	-0.681	-	-1.321	-
9.	+0.508	-0.530	-0.281	+0.391	-0.296	+0.175
10.	-0.136	+0.642	+0.917	+1.012	-1.878	1.288
11.	+0.908	+2.100	+0.450	-0.473	+1.021	+4.099*
12.	-	+0.991	-	+1.432	-	-1.000
13.	-1.923	-2.152*	-0.726	+0.249	-1.591	-0.670
14.	+0.077	-1.076	+1.424	+1.496	+2.214	+0.861
15.	-	-1.866	-	-3.920*	-	+1.187
16.	-0.952	+0.436	+0.514	-1.068	-0.876	-0.761
17.	-0.151	-0.084	-0.370	+1.121	-0.909	+0.539
18.	-0.240	+0.388	+0.462	+0.179	-0.473	+0.499
19.	-1.614	-	-0.435	-	-0.048	-
20.	+0.407	-0.328	+1.395	+1.208	+0.413	-0.116
21.	-0.419	+0.412	+0.122	+1.075	-0.512	+0.246
22.	+1.004	-1.653	-0.701	+0.373	+0.698	-1.660
23.	+0.219	0.000	+0.127	+0.578	+1.930	+1.417
24.	+0.750	-0.971	+1.049	+0.565	+2.544*	0.000
25.	-0.267	-0.017	-1.150	+0.617	+0.475	-0.470

Rough vs smooth awns: M2 GPxSl38-1000rads

	Height t & p		Tiller No t & p		Ear Length t & p	
	rep 1	rep 2	rep 1	rep 2	rep 1	rep 2
1.	-0.387	-	+0.456	-	+0.464	-
2.	-	-0.052	-	+1.207	-	+0.326
3.	+0.525	+0.293	+0.517	-0.606	+0.986	+0.756
4.	-0.254	-	-1.516	-	-1.510	-
5.	-0.197	-1.264	-1.130	+1.586	+1.262	+0.637
6.	-	-	-	-	-	-
7.	-1.277	-1.606	-2.134	-0.524	-4.088***	-0.764
8.	-2.124	-0.143	+1.231	+0.901	-0.069	+0.489
9.	+0.248	-0.346	+1.996	+1.111	+0.848	+0.276
10.	-0.222	-0.265	+0.446	0.000	+1.263	+0.378
11.	+0.575	-1.314	-0.430	+0.384	+0.129	-1.663
12.	-	-	-	-	-	-

Rough vs smooth awns: M2 SL38xGP-500rads

	Height t & p		Tiller No t & p		Ear Length t & p	
	rep 1	rep 2	rep 1	rep 2	rep 1	rep 2
1.	-0.757	+0.948	+1.410	-0.247	+1.536	+1.122
2.	-	+2.792*	-	-4.279 ***	-	+0.397
3.	+0.409	+0.173	+1.566	-0.626	+0.181	+1.547
4.	-2.212*	-1.062	+0.948	+1.716	-1.407	-0.231
5.	-1.180	+0.700	+0.556	+1.749	-0.860	+0.611
6.	-0.617	-1.031	-2.678*	+0.973	-2.599*	-0.052
7.	-	+0.825	-	+1.226	-	-0.117
8.	-	-	-	-	-	-
9.	-1.245	-1.043	-1.579	-1.486	-0.412	-1.096
10.	-0.581	-2.114	-0.218	+0.106	+0.637	-0.580
11.	-0.350	-1.157	-1.388	-1.507	+0.847	-1.543
12.	-1.455	-0.790	+1.352	+0.989	-0.217	+0.195
13.	-0.365	+0.533	-1.476	+0.662	-0.440	+1.466
14.	+0.745	-	+0.049	-	+3.589***	-
15.	+0.998	+0.820	+0.327	+0.667	+1.323	+0.396
16.	+0.123	-0.001	-1.528	+1.859	-1.337	+0.735
17.	-0.324	+1.577	+1.718	-2.061	-0.378	+2.464*
18.	-1.037	+0.915	+0.349	+1.208	+1.995	-0.766

Rough vs smooth awns: M₂ S138xGP-1000rads

	Height t & p		Tiller No t & p		Ear Length t & p	
	rep 1	rep 2	rep 1	rep 2	rep 1	rep 2
1.	+0.374	+0.904	-0.613	+1.138	-0.512	+1.350
2.	-0.730	-0.126	-0.528	+0.302	-0.972	+0.606
3.	-0.955	+0.340	+1.102	+0.060	+0.361	+0.882
4.	+0.749	-	-0.259	-	+1.049	-
5.	+1.340	+0.605	+1.671	-0.967	+2.580*	-0.467
6.	+0.572	-0.110	+0.788	+0.439	+1.559	-0.271
7.	-	+1.339	-	+2.180	-	+0.693

Tall vs dwarf: M₂ GPxS138-500rads

	Tiller rep 1	No t & p rep 2
1.	-0.917	+0.186
2.	+1.707	+0.296
3.	+1.667	+0.128
4.	-1.182	+1.445
5.	+0.313	-
6.	+0.106	-2.017
7.	+1.335	-
8.	+0.818	-
9.	-	+0.028
10.	+1.938	+0.815
11.	+0.881	-
12.	-2.432*	+0.276
13.	-1.456	+0.354
14.	-	-
15.	+0.478	-
16.	+0.227	+0.245
17.	-1.435	-0.800
18.	+0.560	-0.165
19.	+1.128	-
20.	+0.257	-0.251
21.	-0.841	-1.212
22.	+0.233	+0.299
23.	+0.798	+0.667
24.	-0.448	-0.096
25.	-0.486	+1.567

Tall vs dwarf: M2 GPxSl38-1000rads

	Tiller No rep 1	t & p rep 2
1.	+1.188	+1.022
2.	+0.654	+1.876
3.	+1.777	+1.186
4.	-	-
5.	-0.051	+0.381
6.	-0.560	+0.309
7.	+1.038	+0.546
8.	-0.390	+1.157
9.	-0.149	+1.017
10.	+0.305	-0.240
11.	-2.178*	+0.396
12.	-0.976	+1.353

Tall vs dwarf: M2 S138xGP-500rads

	Tiller No rep 1	t & p rep 2
1.	-0.181	-
2.	+0.874	-
3.	+1.114	-1.907
4.	+0.487	-2.333*
5.	-0.378	0.000
6.	+1.276	+0.312
7.	+1.968	+1.306
8.	+0.111	-0.486
9.	+0.306	-0.692
10.	+0.817	+2.038
11.	-2.070	+1.294
12.	+0.031	-2.430*
13.	+1.255	+1.410
14.	-0.483	-0.039
15.	+0.501	+0.266
16.	+0.435	-
17.	+0.353	-1.239
18.	-0.819	-0.081

Tall vs dwarf: M2 S138xGP-1000rads

	Tiller No rep 1	t & p rep 2
1.	-0.294	+0.724
2.	+0.281	+0.805
3.	-0.376	+0.735
4.	+0.690	-
5.	-1.037	-0.207
6.	+0.847	+1.593
7.	+0.844	+1.150

Long vs short rachilla hairs: M₂ GPxS138-500rads

	Height t & p		Tiller No t & p		Ear Length t & p	
	rep 1	rep 2	rep 1	rep 2	rep 1	rep 2
1.	-0.647	-0.493	+0.462	+0.641	+0.663	-0.430
2.	-0.660	-	-0.132	-	-0.060	-
3.	-0.028	-1.366	+0.367	-1.227	-0.874	-1.520
4.	-0.792	-0.168	+0.360	-0.484	-0.648	-0.042
5.	+0.616	-1.228	+0.599	+0.061	+1.076	-0.241
6.	-0.892	+0.727	+0.149	+0.885	-1.154	+2.071
7.	-0.969	-	-2.947**	-	-1.592	-
8.	-0.708	-	-0.681	-	-1.321	-
9.	-0.249	-1.503	+1.836	-0.913	+2.340*	-0.335
10.	-1.346	-	-1.102	-	-1.150	-
11.	-0.010	-	+0.734	-	+0.349	-
12.	-0.124	-0.526	+0.161	-0.381	-2.062	-0.755
13.	+0.167	-0.146	+0.726	+0.512	+0.488	-1.688
14.	+0.077	+2.165	+1.513	+0.907	+2.214	+1.531
15.	-0.578	-2.903*	+1.401	+1.366	+0.170	-0.488
16.	-1.267	+0.504	+2.708*	-0.063	-0.939	-1.359
17.	-0.941	-	+0.796	-	+1.061	-
18.	-0.605	-0.977	-0.016	+1.544	-0.889	+1.991
19.	-1.614	-	+1.217	-	+0.991	-
20.	0.000	-0.859	+1.971	+1.727	-0.308	+1.041
21.	+0.142	-0.164	+1.445	+1.204	+1.700	+1.738
22.	+1.014	-	+0.566	-	+0.666	-
23.	-0.858	-0.514	+0.256	+0.868	+0.470	+1.418
24.	-0.512	-1.414	+8.954***	-1.545	+0.496	+0.241
25.	-1.674	-0.151	+0.133	+1.947	-0.970	+0.605

Long vs short rachilla hairs: M2 GPxSl38-1000rads

	Height t & p		Tiller No t & p		Ear Length t & p	
	rep 1	rep 2	rep 1	rep 2	rep 1	rep 2
1.	+0.043	-	+1.090	-	+0.918	-
2.	-	-0.418	-	+1.850	-	+0.940
3.	-	-0.714	-	-0.291	-	+0.248
4.	-0.890	-	-2.338*	-	-2.029	-
5.	-0.512	-0.520	+0.254	-0.185	+0.085	+0.894
6.	-0.757	-	+0.928	-	+1.117	-
7.	-2.457*	-1.472	-1.955	-8.029***	-1.762	-1.988
8.	-0.314	-	+1.668	-	+0.905	-
9.	-0.140	-0.714	+1.538	+0.429	-0.685	+0.880
10.	-1.810	-	-0.547	-	-1.726	-
11.	-0.316	-0.944	+0.649	+1.388	+2.049	+0.309
12.	-	-	-	-	-	-

Long vs short rachilla hairs: M2 S138xGP-500rads

	Height t & p		Tiller No t & p		Ear Length t & p	
	rep 1	rep 2	rep 1	rep 2	rep 1	rep 2
1.	-1.442	-	+0.853	-	-0.616	-
2.	-	-1.970	-	+0.856	-	-1.090
3.	-1.998	+0.309	+0.555	+1.811	+0.634	+0.100
4.	-0.852	-0.222	+1.111	+1.431	+1.471	+0.526
5.	-0.001	-2.986**	+1.123	-1.217	+0.668	-3.371***
6.	-1.455	-1.219	+1.358	+0.989	-0.069	+0.751
7.	-	+0.320	-	+1.732	-	+1.046
8.	-0.012	-0.704	+2.046	-0.331	+2.840*	-0.253
9.	+0.668	+0.922	+0.945	-0.944	+0.885	-0.176
10.	-1.921	-1.493	-0.609	+0.227	-0.610	-0.036
11.	-0.629	+0.378	-0.919	+0.048	+0.434	+2.797*
12.	-1.283	-1.684	-0.082	+1.193	-0.355	-1.859
13.	-2.713*	+1.771	+0.506	-7.185***	-0.451	+0.541
14.	-0.677	-2.235*	+1.818	+1.457	+0.644	-0.267
15.	-0.476	+0.246	+1.635	+2.030	+1.190	+0.107
16.	-1.655	-1.256	+0.190	+0.367	-0.935	0.000
17.	-0.729	-1.541	-0.001	+1.293	-0.716	-0.619

Long vs short rachilla hairs: M₂ S138xGP-1000rads

	Height t & p		Tiller No t & p		Ear Length t & p	
	rep 1	rep 2	rep 1	rep 2	rep 1	rep 2
1.	-0.396	-	+0.258	-	-0.861	-
2.	-0.702	+0.038	-0.528	+1.935	-0.898	+1.511
3.	-	-1.351	-	+0.472	-	-0.771
4.	+0.154	-	-1.091	-	-0.900	-
5.	+0.601	-1.547	+1.919	+0.756	+0.760	-0.735
6.	+0.090	-1.758	+0.867	+0.098	+1.306	-0.470
7.	+0.440	+0.070	+1.685	+4.833***	+1.590	+0.132

Table B1: Analysis of variance of data from the F_2 / M_2 generations in the barley irradiated female study

Key:

= non-significant

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.005$

[For each parent, any 'within families' variation must have been environmental in origin since individuals were genetically identical. So the results for both parents were pooled to estimate the environmental mean square for each character. Each within families mean square was tested against its environmental mean square to confirm that there were genetic differences among individuals for each trait.

Since both main effects (families, reps) in this analysis were random, the appropriate comparison for the between families mean square (MS) was, where it was significant, the interaction MS. Where it was not significant, the interaction MS provided an estimate of error that could be pooled with the within families MS and used to test the significance of the between family MS.]

GP	within families		
	\bar{x}	MS	df
height	60.05	11.694	36
tiller number	5.31	6.411	36
ear length	8.03	0.658	36
awn length	12.76	1.114	36
green tiller number	2.25	3.311	36

between families

error

MS	df	MS	df	VR
66.0935	2	43.7111	4	1.51
4.8225	2	6.2188	40	0.78
0.0675	2	0.7280	40	0.09
0.1985	2	1.0877	40	0.18
3.0890	2	3.1521	40	0.98

		within families	
4082	\bar{x}	MS	df
height	86.13	31.175	36
tiller number	3.11	4.722	36
ear length	6.77	0.669	36
awn length	17.80	1.983	36
green tiller number	1.00	2.744	36

between families		error		
MS	df	MS	df	VR
125.3645	2	158.3335	4	0.79
0.2890	2	4.6754	40	0.06
0.1180	2	0.7530	40	0.16
0.9475	2	2.0974	40	0.45
1.8000	2	2.8796	40	0.63

F ₂	\bar{x}	within families		environment		VR
		MS	df	MS	df	
height	79.02	94.412	120	21.434	72	4.40
tiller number	5.65	16.457	120	5.567	72	2.96
ear length	7.80	1.616	120	0.664	72	2.43
awn length	17.65	6.802	120	1.549	72	4.39
green tiller number	2.00	4.407	120	3.028	72	1.46

between
families

error

MS

df

MS

df

VR

*** 160.7060 9 287.4665 18 0.56

*** 20.6345 9 16.8331 138 1.23

*** 2.4275 9 1.747 138 1.39

*** 6.5590 9 8.5234 138 0.77

* 3.2300 9 4.2966 138 0.75

M ₂ -3Krad	\bar{x}	within families		environment		VR
		MS	df	MS	df	
height	82.96	130.074	196	21.434	72	6.07
tiller number	5.70	29.098	196	5.567	72	5.23
ear length	7.78	1.821	196	0.664	72	2.74
awn length	18.09	7.261	196	1.549	72	4.69
green tiller number	1.89	3.857	196	3.028	72	1.27

	between families		error			
	MS	df	MS	df	VR	
***	431.1180	15	138.0389	226	3.12	***
***	36.8735	15	28.0236	226	1.32	
***	3.6425	15	1.8159	226	2.01	*
***	13.3980	15	6.9745	226	1.92	*
	5.6690	15	3.9239	226	1.44	

M2-4Krad	x	within families		environment		VR
		MS	df	MS	df	
height	80.25	164.076	114	21.434	72	7.65
tiller number	5.59	13.603	114	5.567	72	2.44
ear length	7.58	2.661	114	0.664	72	4.01
awn length	17.69	8.836	113	1.549	72	5.71
green tiller number	2.15	4.834	114	3.028	72	1.60

between
families

error

MS

df

MS

df

VR

*** 197.7660 9 185.7416 132 1.06

*** 15.7345 9 12.9693 132 1.24

*** 4.1155 9 2.6091 132 1.58

*** 9.2540 9 8.9702 131 1.03

* 4.6350 9 4.7133 132 0.98

M ₂ -5Krad	within families			environment		
	\bar{x}	MS	df	MS	df	VR
height	79.83	128.847	32	21.434	72	6.01
tiller number	5.02	8.925	32	5.567	72	1.60
ear length	7.70	1.019	32	0.664	72	1.53
awn length	17.20	7.775	32	1.549	72	5.02
green tiller number	1.97	4.936	32	3.028	72	1.63

between
families

error

MS

df

MS

df

VR

***	66.6665	2	123.7780	36	0.54	
***	15.5600	2	10.1877	36	1.53	
***	5.0680	2	1.0594	36	4.78	*
***	9.8765	2	7.8956	36	1.25	
*	2.1500	2	4.9172	36	0.44	

M ₂ -6Krad	\bar{x}	within families		environment		VR
		MS	df	MS	df	
height	80.29	100.915	92	21.434	72	4.71
tiller number	5.67	10.191	92	5.567	72	1.83
ear length	7.60	1.247	92	0.664	72	1.88
awn length	17.81	5.613	92	1.549	72	3.62
green tiller number	2.45	4.3652	92	3.028	72	1.44

between
families

error

MS

df

MS

df

VR

*** 44.2855 7 110.8560 106 0.40

*** 11.7235 7 10.1479 106 1.16

*** 2.2180 7 1.3124 106 1.69

*** 6.4825 7 11.0640 14 0.59

6.9335 7 8.0006 14 0.87

		within families		environment		
M ₂ -7Krad	\bar{x}	MS	df	MS	df	VR
height	80.20	103.662	40	21.434	72	4.84
tiller number	7.16	14.045	40	5.567	72	2.52
ear length	7.95	1.424	40	0.664	72	2.14
awn length	19.19	3.870	40	1.549	72	2.50
green tiller number	2.53	5.563	40	3.028	72	1.84

	between families		error			
	MS	df	MS	df	VR	
***	168.6845	4	117.4061	48	1.44	
***	48.6648	4	43.6165	8	1.12	
***	3.6950	4	5.6821	8	0.65	
***	17.0945	4	3.2315	48	5.29	***
*	9.4950	4	5.0740	48	1.82	

M ₂ -8KradS	\bar{x}	within families		environment		VR
		MS	df	MS	df	
height	82.93	66.008	12	21.434	72	3.08
tiller number	6.67	43.600	12	5.567	72	7.83
ear length	8.03	2.600	12	0.664	72	3.92
awn length	17.50	8.317	12	1.549	72	5.37
green tiller number	2.45	7.200	12	3.028	72	2.38

between
families

error

MS

df

MS

df

VR

-

-

-

-

-

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**Table B2: Major gene frequencies in the second generation of the barley
irradiated females study**

Key to genotypes

- | | | | |
|-----|--------|-----|--------|
| 1. | RVOSN | 17. | RVosn |
| 2. | RVOSn | 18. | RvOSn |
| 3. | RVOsN | 19. | rVOSn |
| 4. | RVoSN | 20. | RvosN |
| 5. | RvOSN | 21. | rVosN |
| 6. | rVOSN | 22. | rvoSN |
| 7. | RVOsn | 23. | rvOsN |
| 8. | RVoSsn | 24. | rvOSsn |
| 9. | RvOSsn | 25. | RvoSsn |
| 10. | rVOSsn | 26. | rVoSsn |
| 11. | RVosN | 27. | Rvosn |
| 12. | RvOsN | 28. | rVosn |
| 13. | rVOSn | 29. | rvOsn |
| 14. | RvoSN | 30. | rvoSsn |
| 15. | rVoSN | 31. | rvosN |
| 16. | rvOSN | 32. | rvosn |

GENOTYPE

Pooled F ₂	117	63	26	51	37	26	6	24	9	10	11	12	13	14	15	16
	2	7	11	4	7	2	8	3	6	3	1	3	2	-	3	2
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32

M₂-3Krad

(1)	7	3	1	5	4	7	1	-	1	-	4	-	4	1	1	-
1	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
(2)	4	1	1	1	17	3	-	1	5	-	-	4	2	6	1	1
-	-	1	-	-	-	-	2	-	1	-	-	-	1	-	-	1

(3)	6	3	-	9	-	-	-	1	-	-	3	-	-	2	2	-
3	-	-	-	2	1	-	-	-	3	-	-	-	-	-	-	1

M₂-3KradS

(9)	9	2	1	3	6	1	2	5	2	-	2	3	3	2	-	2
	-	1	1	-	1	-	-	1	-	-	-	-	-	-	-	-
(10)	1	-	-	-	-	1	-	-	1	1	-	1	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
(11)	14	3	1	7	7	1	-	-	2	-	1	-	2	1	-	1
	-	-	1	-	1	-	2	-	-	2	1	-	-	-	-	-
(12)	10	7	2	4	3	1	2	3	4	1	-	3	2	5	-	1
	2	-	2	-	-	-	-	-	1	-	-	1	-	1	-	-
(13)	13	4	3	3	8	1	-	-	1	-	1	4	5	2	1	1
	-	-	2	-	1	-	2	-	-	-	1	1	1	-	-	-

M_2 -3KradS

(14)	15	4	3	2	5	-	1	-
	-	-	-	-	1	-	1	-

(15)	7	4	1	3	3	5	3	1
	1	-	-	-	1	-	-	-

(16)	8	3	1	6	3	1	2	5
	1	-	2	-	1	-	1	-

(17)	17	6	6	3	4	1	1	2
	-	-	1	-	2	-	1	1

1	-	1	-	2	1	1	-
-	-	-	-	-	-	-	-
-	2	-	-	2	2	-	1
1	-	-	-	-	-	-	-
1	2	-	-	4	-	1	-
-	-	-	-	2	-	-	-
1	-	-	-	3	2	-	-
1	-	-	-	-	-	-	-

M₂-4KradS

(1)	4	3	-	7	8	1	1	4	3	1	-	1	-	1	-
	-	-	1	-	1	-	-	1	3	1	-	2	-	-	-
(2)	10	2	3	3	8	1	4	1	2	1	1	-	3	1	-
	1	-	2	-	1	-	1	-	-	2	-	-	-	-	1
(3)	7	9	1	5	5	2	1	-	-	-	3	1	3	2	-
	1	-	1	-	2	-	2	-	-	-	-	-	1	-	1
(4)	10	5	1	8	4	5	2	5	2	-	1	1	1	-	5
	1	-	1	2	-	-	-	-	-	-	1	2	1	-	-
(5)	5	5	2	1	8	-	-	-	1	-	-	-	-	1	-
	-	-	-	-	-	-	1	-	-	1	-	-	-	-	-

M₂-4KradS

(6)	5	4	1	5	3	1	1	3	-	1	-	2	1	-	2
	-	2	-	-	1	-	3	-	1	-	-	-	-	1	-
(7)	11	2	1	6	3	4	-	-	2	1	-	2	3	2	1
	1	-	3	1	2	-	2	1	-	-	-	1	1	-	-
(8)	6	3	-	7	3	1	1	3	1	1	2	1	5	2	3
	-	-	1	1	2	1	-	-	1	1	-	-	-	-	-
(9)	14	2	3	6	4	1	1	1	1	1	1	-	4	2	-
	-	-	-	1	4	1	-	-	-	-	-	-	-	-	1
(10)	1	9	-	-	-	-	2	3	1	1	1	1	-	-	-
	-	1	3	-	-	-	-	-	1	-	1	-	-	-	-

M₂-5Krad

(1)	10	1	3	4	1	1	-	-	3	-	1	1	4	2	1	1
	2	-	1	-	-	1	1	-	-	-	-	1	-	-	-	-

(2)	3	2	2	1	3	1	-	-	2	-	1	-	-	1	-	-
	-	-	-	2	-	1	1	-	1	-	2	-	-	1	-	-

(3)	10	5	2	2	8	1	5	2	2	2	2	-	6	-	1	1
	1	1	3	-	1	-	-	-	1	-	-	-	-	1	-	-

M₂-6KradS

(1)	13	8	2	9	2	2	2	2	1	-	2	-	1	1	-	1
	-	-	3	-	-	3	1	-	-	-	-	-	-	-	-	1
(2)	14	-	-	2	3	-	-	-	1	-	-	1	1	3	-	-
	-	-	-	1	1	-	-	-	1	-	-	1	1	-	1	-
(3)	14	3	2	6	8	2	-	1	4	-	1	2	1	4	-	-
	-	-	-	-	-	1	-	-	1	-	-	1	1	-	-	-
(4)	14	6	1	3	6	2	2	2	2	-	-	-	2	3	-	-
	1	-	1	-	2	-	1	-	1	1	-	-	-	-	1	-
(5)	9	3	3	6	7	1	3	3	-	-	2	-	2	-	1	-
	-	-	1	-	-	-	-	-	1	2	-	-	-	-	-	-

M₂-6KradS

(6)	1	-	-	-	3	11	-	-	5	-	1	1	2	4
-	-	-	2	-	1	2	4	2	1	-	1	-	1	-

(7)	1	4	2	6	12	1	2	-	2	-	4	-	5	-
3	1	-	-	1	1	-	-	-	3	-	1	1	-	2

(8)	6	2	2	1	3	1	-	1	-	1	2	1	-	-
-	-	-	-	-	1	-	-	1	-	-	-	-	-	-

Table B3: χ^2 analysis of M_2 families whose major gene frequencies differed significantly from those of the F_2 in the barley irradiated females study

Key:

= non-significant

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.005$

R:r χ^2 P

S:s χ^2 P

pooled F_2

399:118

391:126

M_2 -3KradS

(2)

(3)

(5)

(8)

(9)

(10) 2:4 4.29 *

(12)

(13)

34:21 5.66 *

V:v χ^2 P

O:o χ^2 P

N:n χ^2 P

395:122

364:153

365:152

15:38 67.99 ***

9:27 35.59 ***

25:20 4.91 *

35:6 4.41 *

30:17 4.12 *

32:24 4.89 *

30:20 4.97 *

R:r χ^2 P

S:s χ^2 P

M₂-4KradS

(1)

(4)

(7) 32:19 6.05 *

(8)

(9)

(10)

(pooled)

307:124 4.47 *

M₂-5KradS

(2)

(3)

36:21 4.78 *

(pooled)

77:43 8.50 ***

V:v chi² P

O:o chi² P

N:n chi² P

27:17 5.53 *

24:20 5.32 *

24:20 5.46 *

30:25 6.63 **

23:23 9.20 ***

42:6 6.60 *

3:22 38.59 ***

10:14 16.08 ***

R:r χ^2 P S:s χ^2 P V:v χ^2 P O:o χ^2 P N:n χ^2 P

M_2 -6Krad

(2)			19:12	3.92 *	
(3)	48:6	4.19 *	33:21	7.01 **	
(6)	5:37	101.67 ***	24:18	8.65 ***	
(7)	49:6	4.42 *	34:21	5.66*	31:24 5.20 *

M_2 -7Krad

(1)	19:16	8.62***	31:4	4.71 *	18:17	6.20 *
(2)	1:5	8.37***				
(3)	7:9	8.82***	0:19	41.92 ***	6:13	13.92 ***
..(4)						
(5)	29:18	4.92*				
(pooled)	68:55	27.53***				

	RS : Rs : rS : rs	chi ²	P	all genes	chi ²	df	P
pooled F ₂	317 : 66 : 60 : 55	-	-	(See appendix B2)	-	-	-

M₂-3KradS

(2)					83.03	18	***
(3)					42.38	15	***
(7)	30 : 4 : 4 : 12	9.51	*		29.67	18	*
(13)		8.67	*				

M₂-4KradS

(1)					36.05	16	***
(2)					30.22	18	*
(7)	26 : 5 : 7 : 12	9.33	*				
(10)							
(pooled)	262 : 55 : 44 : 65	8.39	*		38.72	13	***

RS : Rs : rs : rs χ^2 P all genes

χ^2 df P

M_2 -5KradS

(1)	64 : 25 : 13 : 18	9.03	*	28.22	16	*
(2)				30.11	12	***
(3)				38.37	19	**
(pooled)				55.36	24	***

M_2 -6KradS

(6)	5 : 0 : 27 : 10	125.22	***	106.03	15	***
(7)	33 : 15 : 1 : 5	13.36	***	89.47	18	***

M_2 -7KradS

(1)	19 : 6 : 0 : 9	12.29	***	48.55	9	***
(3)						
(4)	6 : 3 : 1 : 5	8.98	*			
(pooled)	62 : 28 : 6 : 24	24.97	***	65.51	23	***

**Table B4: Correlation analysis of the second generation data from the
the barley irradiated females study**

Key:

= non-significant

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.005$

To test the hypothesis that all r s are estimates of the same ρ :-

$$\chi^2 = \frac{\sum (n_i - 3) z_i^2 - [\sum (n_i - 3) z_i]^2}{\sum (n_i - 3)}$$

To test the significance of the difference between r and ρ :-

$$F = (z(r) - z(\rho))^2 / \sigma^2(z)$$

$$\sigma^2(z) = 1/(n-3)$$

To combine independent estimates of ρ :-

$$z(\rho) = \sum (n_i - 3) z_i / \sum (n_i - 3)$$

F ₂	family	n	height & tiller number			height & ear length			height & awn length		
			Z	F	P	Z	F	P	Z	F	P
	1	15	.1917	-	-	.7421	-	-	1.0247	-	-
	2	15	.1162	-	-	.1400	-	-	.5108	-	-
	3	15	.6199	-	-	.7077	-	-	.7371	-	-
	4	15	.0981	-	-	.1308	-	-	1.0161	-	-
	5	15	.0446	-	-	-.0304	-	-	.9793	-	-
	6	15	.1555	-	-	.8184	-	-	.7832	-	-
	7	15	.0578	-	-	-.0286	-	-	.0257	-	-
	8	15	.2548	-	-	.4761	-	-	.8431	-	-
	9	15	.0999	-	-	.4993	-	-	.6780	-	-
	10	15	.4954	-	-	-.0316	-	-	.1694	-	-

$\chi^2 = 4.06$
 P = NS

$\chi^2 = 12.77$
 P = NS

$\chi^2 = 12.95$
 P = NS

M ₂ -3Krad		height & tiller number				height & ear length				height & awn length			
		n	Z	F	P	Z	F	P	Z	F	P		
family	1	15	.0706	0.24		.0778	0.84		.2648	2.04			
	2	15	-.1035	1.21		.3746	0.01		.5967	0.08			
	3	15	.4363	0.06		.9114	3.89	*	1.1070	2.22			
	4	15	.3637	0.27		.1061	0.67		.6929	0.00			
	5	15	-.3686	4.06	*	.5925	0.75		.7934	0.16			
	6	15	-.3993	4.51	*	.4388	0.11		1.0289	1.49			
	7	15	.2144	0.00		.5760	0.65		-.1624	8.45	***		
	8	15	.3451	0.21		.6039	0.82		.9143	0.68			
	9	15	.0172	0.64		.7894	2.40		1.1520	2.71			
	10	15	.6761	0.43		.5894	0.12		1.7489	2.30			
	11	15	.3965	0.40		.9154	3.94	*	.4190	0.80			
	12	15	-1.2232	24.77	***	.4678	0.19		.5810	0.11			

M ₂ -3Krad	n	height & tiller number		height & ear length		height & awn length	
		Z	F	P	Z	F	P
family 13	15	.4418	0.63		1.2837	10.63	***
14	15	.2485	0.01		.9976	5.15	*
15	15	.5725	1.55		.7629	2.12	
16	15	.6707	2.51		.6588	1.20	
17	15	.2816	2.94		.2164	0.19	
					1.4177	6.59	*
					.9652	1.00	
					.3984	0.93	
					1.0483	1.66	
					.9161	0.69	

chi² = 42.08
P = ***

chi² = 19.74
P = NS

chi² = 30.26
P = *

M ₂ -4Krad	n	height	tiller number	
		Z	F	P

family 1	15	-.0921	1.12	
2	15	.1822	0.01	
3	15	.1520	0.05	
4	15	.0602	0.28	
5	13	1.0048	6.26	*
6	15	.0261	0.42	
7	15	.1550	0.04	
8	15	.3744	0.31	
9	15	.2233	0.00	
10	15	-.0454	0.67	

$$\chi^2 = 9.37$$

P = NS

height & ear length

Z F P

.5726	0.64	
.8219	2.76	
.2919	0.03	
-1.3876	35.91	***
.8437	2.51	
.3522	0.00	
.3744	0.01	
.6718	1.30	
.3273	0.00	
.4979	0.17	

$$\text{chi}^2 = 43.29$$

P = ***

height & awn length

Z F P

.8775	2.41	
1.0816	1.97	
.7765	0.12	
1.4003	6.28	*
1.3235	4.17	*
.8776	0.48	
.4332	0.71	
1.3346	5.19	*
.8183	0.24	
.7547	0.04	

$$\text{chi}^2 = 9.92$$

P = NS

M ₂ -5Krad	n	height & tiller number		height & ear length		height & awn length	
		Z	F	P	Z	F	P
family 1	15	-.1525	1.61		.4938	0.28	1.2260
2	10	.2866	0.04		.2557	0.05	1.2381
3	15	-.2898	3.04		.5134	0.35	.7956

$\chi^2 = 1.51$
 P = NS

$\chi^2 = 0.34$
 P = NS

$\chi^2 = 1.39$
 P = NS

*

M ₂ -6KradS	n	height & tiller number		
		Z	F	P

family 1	15	.2782	0.05
2	10	-.0884	0.64
3	15	.1991	0.00
4	15	.3052	0.10
5	15	-.1684	1.75
6	15	.0129	0.48
7	15	.1760	0.02
8	15	.3506	0.23

$$\chi^2 = 2.85$$

P = NS

height & ear length

Z F P

.3131	0.01	
.6188	0.53	
.4545	0.15	
.5034	0.31	
.3326	0.00	
.3141	0.01	
.3654	0.01	
.0106	1.32	

height & awn length

Z F P

.8985	0.59	
.2586	1.22	
.9479	0.88	
.6209	1.96	
-.0364	6.10	*
.8963	0.58	
.7890	0.15	
1.1198	2.36	

$\chi^2 = 2.34$
P = NS

$\chi^2 = 11.82$
P = NS

height & tiller number

M_2 -7KradS	n	Z	F	P
---------------	---	---	---	---

family 1	15	.7816	3.87	*
2	5	-.0851	0.18	
3	8	.3544	0.10	
4	8	.7357	1.36	
5	15	.1725	0.02	

$$\chi^2 = 3.26$$

P = NS

height & ear length

Z F P

.9721	4.76	*
-.2207	0.63	
.5371	0.19	
.9858	2.07	
.7557	2.05	

height & awn length

Z F P

.5788	0.12	
-.5357	2.94	
.4452	0.27	
1.6401	4.64	*
.6517	0.01	

$\chi^2 = 2.96$

P = NS

$\chi^2 = 7.97$

P = NS

tiller number & ear length tiller number & awn length ear length & awn length

F₂

n

Z

F

P

Z

F

P

Z

F

P

family 1

15

.6197

-

-

.3991

-

-

1.5828

-

-

2

15

.6555

-

-

.4745

-

-

.6021

-

-

3

15

1.1123

-

-

.7471

-

-

1.1516

-

-

4

15

.5149

-

-

.3239

-

-

.4576

-

-

5

15

.4080

-

-

-.0408

-

-

.3638

-

-

6

15

.1109

-

-

.1678

-

-

.4508

-

-

7

15

.8894

-

-

.3857

-

-

.6856

-

-

8

15

.5828

-

-

.5652

-

-

.8509

-

-

9

15

.1944

-

-

.5496

-

-

.7615

-

-

10

15

.6254

-

-

.2551

-

-

.0855

-

-

chi² = 9.48

P = NS

chi² = 8.43

P = NS

chi² = 19.56

P = *

M ₂ -3Krad	family	n	tiller number & ear length			tiller number & awn length			ear length & awn length		
			Z	F	P	Z	F	P	Z	F	P
	1	15	.6135	0.02		.4910	0.05		1.0856	-	-
	2	15	-.0562	4.73	*	.2796	0.27		.4186	-	-
	3	15	.2723	1.08		.2485	0.14		1.0225	-	-
	4	15	.1979	1.68		.3096	0.17		.3499	-	-
	5	15	.2517	1.23		.1685	0.82		.7610	-	-
	6	15	.1830	1.81		-.0939	3.29		.8429	-	-
	7	15	.6296	0.04		.4305	0.00		.5980	-	-
	8	15	.1591	2.04		.4653	0.01		.6476	-	-
	9	15	.3862	0.41		-.0068	2.28		.9199	-	-
	10	5	-.2721	1.42		.4281	0.00		.8368	-	-
	11	15	.6540	0.08		.4460	0.00		.5908	-	-
	12	15	-.0606	4.80	*	.0916	3.26		1.1766	-	-

M ₂ -3Krad	n	tiller number & ear length			tiller number & awn length			ear length & awn length		
		Z	F	P	Z	F	P	Z	F	P
family 13	15	.7367	0.32		.3929	0.02		1.1209	-	-
	14	.3267	0.72		.3018	0.20		.6893	-	-
	15	.3570	0.55		.1461	0.96		.7079	-	-
	16	.5829	0.00		.4001	0.01		.9887	-	-
	17	.1959	1.70		-.0887	3.22		.5512	-	-

chi² = 11.59
P = NS

chi² = 7.91
P = NS

chi² = 11.54
P = NS

M ₂ -4Krad	family	n	tiller number & ear length		tiller number & awn length		ear length & awn length	
			Z	F	Z	F	Z	P
	1	15	.1306	2.34	-.0856	3.18	.8970	-
	2	15	.2495	1.25	.4588	0.01	.8173	-
	3	15	.6834	0.15	.3706	0.04	.6795	-
	4	15	.0921	2.76	.0923	1.36	-1.0299	-
	5	13	.9328	1.30	.8732	1.97	1.1403	-
	6	15	.1831	1.81	-.1075	1.24	.7110	-
	7	15	.3410	0.64	.6405	0.53	1.0609	-
	8	15	.1406	2.23	.2694	0.31	1.0276	-
	9	15	.4851	0.09	.4003	0.01	.2940	-
	10	10	.1621	1.17	.0721	0.89	.7509	-

$\chi^2 = 7.54$
 P = NS

$\chi^2 = 9.83$
 P = NS

$\chi^2 = 42.62$
 P = ***

M ₂ -5Krads	family	n	tiller number & ear length			tiller number & awn length			ear length & awn length		
			Z	F	P	Z	F	P	Z	F	P

1	15	.4080	0.14		-.0364	2.60		.5133	-		-
2	10	1.2802	4.08	*	.7180	0.58		.7196	-		-
3	15	.1910	1.28		.3282	0.12		.6947	-		-

chi² = 5.49
P = NS

chi² = 2.57
P = NS

chi² = 0.27
P = NS

M ₂ -6Krad		tiller number & ear length			tiller number & awn length			ear length & awn length			
	n	Z	F	P	Z	F	P	Z	F	P	
family	1	15	-.1828	6.83	**	.1144	1.19		.5825	-	-
	2	10	.1370	1.32		.2551	0.21		1.2278	-	-
	3	15	.4760	0.11		.6527	0.60		.7653	-	-
	4	15	.6994	0.20		.4334	0.00		.4896	-	-
	5	15	-.2852	8.81	***	.1116	1.18		.5879	-	-
	6	15	.1141	2.50		.4242	0.00		.3032	-	-
	7	15	-.0024	3.96	*	.2736	0.29		.8870	-	-
	8	15	.6507	0.07		.5502	0.17		.2207	-	-

$\chi^2 = 11.53$
P = NS

$\chi^2 = 3.21$
P = NS

$\chi^2 = 7.01$
P = NS

M ₂ -7Krad		tiller number & ear length				tiller number & awn length				ear length & awn length			
		n	Z	F	P	Z	F	P	Z	F	P		
family	1	15	.9192	1.45		.6573	0.62		1.0805	-	-		
	2	5	.9124	0.23		.6432	0.09		.6017	-	-		
	3	8	.3741	0.20		.3225	0.06		.4510	-	-		
	4	8	.8155	0.30		.5891	0.13		.8855	-	-		
	5	15	.6480	0.07		.4580	0.01		1.0552	-	-		

chi² = 1.24
P = NS

chi² = 0.51
P = NS

chi² = 1.83
P = NS

Table C1: Tuber weights, tuber numbers and plant heights in the irradiated pollen potato study

	Tuber weight		Tuber number		Plant height	
	x	SEM	x	SEM	x	SEM
PI	41.61	± 4.13	3.70	± 0.48	32.60	± 1.36
C	41.57	± 3.35	4.55	± 0.72	20.25	± 1.38
PIxPI	19.12	± 0.94	4.38	± 0.28	23.05	± 1.28
CXC	24.94	± 1.39	4.91	± 0.30	20.82	± 0.66
F ₁ PIXC	42.41	± 3.02	5.26	± 0.36	27.08	± 1.09
M ₁ PIXC-5	27.53	± 3.41	4.47	± 0.69	21.71	± 1.36
M ₁ PIXC-15	23.89	± 2.81	3.50	± 0.54	24.56	± 2.76
F ₂ PIXC/1	14.91	± 1.67	4.14	± 0.37	23.21	± 1.37
2	18.51	± 1.05	2.46	± 0.16	12.08	± 1.63
3	14.08	± 1.23	3.10	± 0.26	11.00	± 1.49
4	20.12	± 1.41	5.03	± 0.40	21.77	± 0.85
5	20.10	± 1.66	3.62	± 0.30	16.46	± 1.58
6	14.60	± 1.39	6.25	± 1.05	22.06	± 2.46
7	16.01	± 1.30	2.38	± 0.18	17.84	± 1.37
8	26.37	± 1.71	5.40	± 0.43	24.65	± 1.25
9	18.91	± 1.39	2.64	± 0.18	12.73	± 1.38
10	14.90	± 1.19	2.78	± 0.20	15.56	± 1.48
11	16.28	± 1.39	3.26	± 0.29	16.05	± 1.93
12	11.80	± 1.23	2.42	± 0.20	9.68	± 1.65
13	17.03	± 1.41	4.12	± 0.28	21.29	± 0.99
M ₂ PIXC-5/1	15.11	± 1.31	3.33	± 0.31	17.91	± 1.27
2	6.78	± 0.70	1.67	± 0.12	-	-
M ₂ PIXC-15/1	10.17	± 1.16	3.77	± 0.37	13.93	± 1.59
2	12.55	± 1.61	3.37	± 0.31	19.84	± 1.60
3	6.57	± 0.99	2.42	± 0.21	10.85	± 1.71

	Tuber weight		Tuber number		Plant height	
	x	SEM	x	SEM	x	SEM
D	43.19	± 4.09	5.00	± 0.62	26.70	± 1.12
C	41.57	± 3.35	4.55	± 0.72	20.25	± 1.38
DXD	25.72	± 1.36	4.51	± 0.26	22.23	± 0.72
CXC	24.94	± 1.39	4.91	± 0.30	20.82	± 0.66
F ₁ DXC	35.42	± 2.08	4.38	± 0.35	22.44	± 0.86
M ₁ DXC-5	27.32	± 2.18	4.18	± 0.33	18.04	± 1.74
M ₁ DXC-10	17.82	± 2.69	3.93	± 0.82	20.14	± 1.63
F ₂ DXC/1	15.31	± 1.30	1.88	± 0.13	9.18	± 2.14
2	25.11	± 1.87	4.22	± 0.34	21.33	± 1.11
3	22.03	± 1.45	4.76	± 0.40	21.56	± 0.98
4	17.09	± 1.74	2.48	± 0.18	11.08	± 1.31
5	12.24	± 1.17	2.28	± 0.18	5.00	± 1.88
6	21.06	± 1.67	5.46	± 0.34	20.46	± 0.87
7	17.78	± 1.99	4.07	± 0.43	19.08	± 1.65
M ₂ DXC-5/1	21.03	± 1.34	3.40	± 0.25	24.73	± 1.07
2	8.16	± 0.94	1.71	± 0.13	-	-
M ₂ DXC-10/1	17.66	± 1.34	3.19	± 0.28	11.12	± 1.46

	Tuber weight		Tuber number		Plant height	
	x	SEM	x	SEM	x	SEM
D	43.19	± 4.09	5.00	± 0.62	26.70	± 1.12
PI	41.61	± 4.13	3.70	± 0.48	32.60	± 1.36
DXD	25.72	± 1.36	4.51	± 0.26	22.23	± 0.72
PIXPI	19.12	± 0.94	4.38	± 0.28	23.05	± 1.28
F ₁ DXPI	40.72	± 2.77	5.39	± 0.41	25.98	± 0.91
M ₁ DXPI	20.01	± 1.85	4.77	± 0.48	21.44	± 1.31
F ₂ DXPI/1	24.61	± 1.77	3.08	± 0.25	24.00	± 1.25
2	23.80	± 1.63	5.06	± 0.37	25.02	± 1.02
3	14.93	± 1.41	2.46	± 0.17	14.75	± 2.15
4	19.47	± 1.41	3.40	± 0.31	22.41	± 1.33
5	17.94	± 1.26	4.10	± 0.45	23.38	± 1.16
6	17.07	± 1.22	4.56	± 0.68	19.96	± 1.02
7	18.51	± 1.44	2.73	± 0.20	11.66	± 1.52
8	22.00	± 1.75	4.32	± 0.40	26.12	± 1.30
9	20.08	± 1.77	3.10	± 0.21	12.28	± 1.52
10	17.63	± 1.63	3.84	± 0.36	18.59	± 1.40
11	16.49	± 1.41	3.98	± 0.41	21.89	± 0.98
12	11.29	± 1.41	2.29	± 0.16	7.83	± 2.04
M ₂ DXPI-5/1	10.23	± 1.26	2.93	± 0.26	12.14	± 1.60
2	7.05	± 1.22	1.48	± 0.10	-	-
3	7.31	± 1.44	3.23	± 0.42	14.67	± 2.27